MAYO CLINIC

Center for Tuberculosis

Laboratory Diagnostics – Whole Genome Sequencing Related to Drug Resistance and Epidemiology

Hind Fadel, MD-PhD
Assistant Professor of Medicine
Mayo Clinic

slide adapted from N. Wengenack, PhD

Accreditation Statement

Accreditation Statement
In support of improving patient care, Mayo Clinic College of Medicine and Science is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC) to provide continuing education for the healthcare team.

Credit Statement(s):

AMA
Mayo Clinic College of Medicine and Science designates this live activity for a maximum of 1.00 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

ACPE
Mayo Clinic College of Medicine and Science designates this educational activity for a maximum of 1.00 ACPE Knowledge-based contact hours. Participants should claim only the credit commensurate with the extent of their participation in the activity.
UAN Number: JA0000238-0000-26-035-L99-P

ANCC
Mayo Clinic College of Medicine and Science designates this activity for a maximum of 1.00 ANCC contact hours. Nurses should claim only the credit commensurate with the extent of their participation in the activity.

This activity was planned by and for the healthcare team, and learners will receive 1.0 Interprofessional Continuing Education (IPCE) credit for learning and change.

Other Healthcare Professionals:
A record of attendance will be provided to all registrants for requesting credits in accordance with state nursing boards, specialty societies or other professional associations.

For disclosure information regarding Mayo Clinic School of Continuous Professional Development accreditation review committee member(s) and staff, please go here to the course accreditation page.

Available Credit
- 1.00 ACPE,
- 1.00 *AMA PRA Category 1 Credit*™
- 1.00 ANCC
- 1.00 Attendance
- 1.00 IPCE

Disclosures

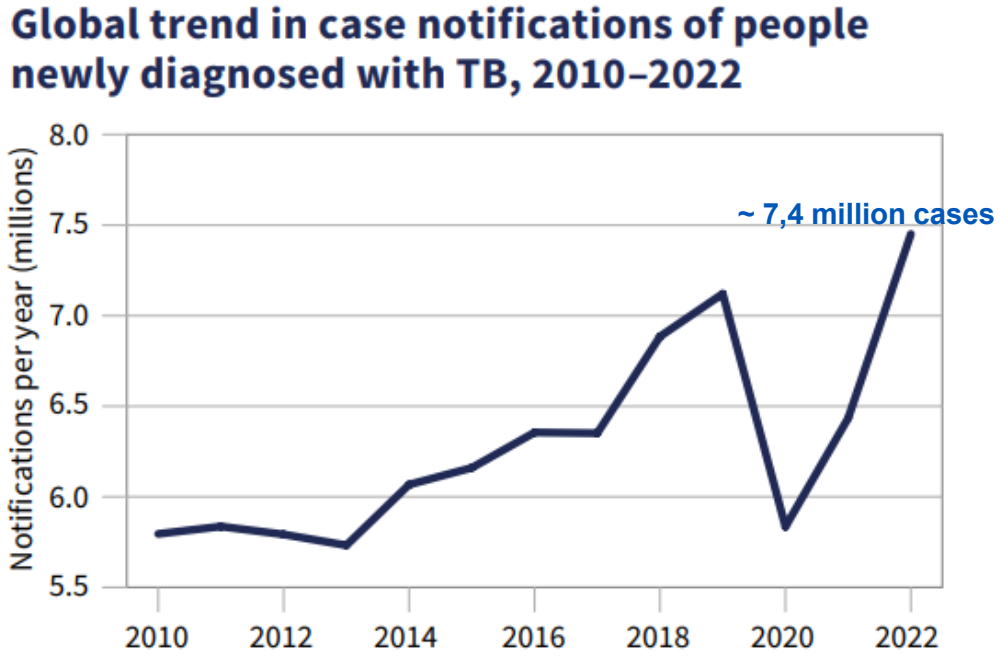
- No relevant financial disclosures to report and no mention of off-label use of any medications or products

Learning Objectives

1. Identify essential tools for TB diagnosis
2. Describe molecular testing methods
3. Explain the role of imaging in TB diagnosis

Global Impact of M. tuberculosis

- Leading cause of death from a single infectious agent
- Each year:
  - 10.6 million cases of active TB worldwide
  - 1.3 million deaths
- >400,000 cases of MDR TB and RR-TB (3.6% of new cases)
- ~ 1.7 billion people have been exposed, don't know extent of latent TB vs cleared infections (1/4 of world population)



6.2 million people diagnosed with pulmonary TB worldwide in 2022
~ 80% of Tb case

WHO: END TB Targets

A WORLD FREE OF TB

ZERO deaths, disease, and suffering due to TB

END THE GLOBAL TB EPIDEMIC

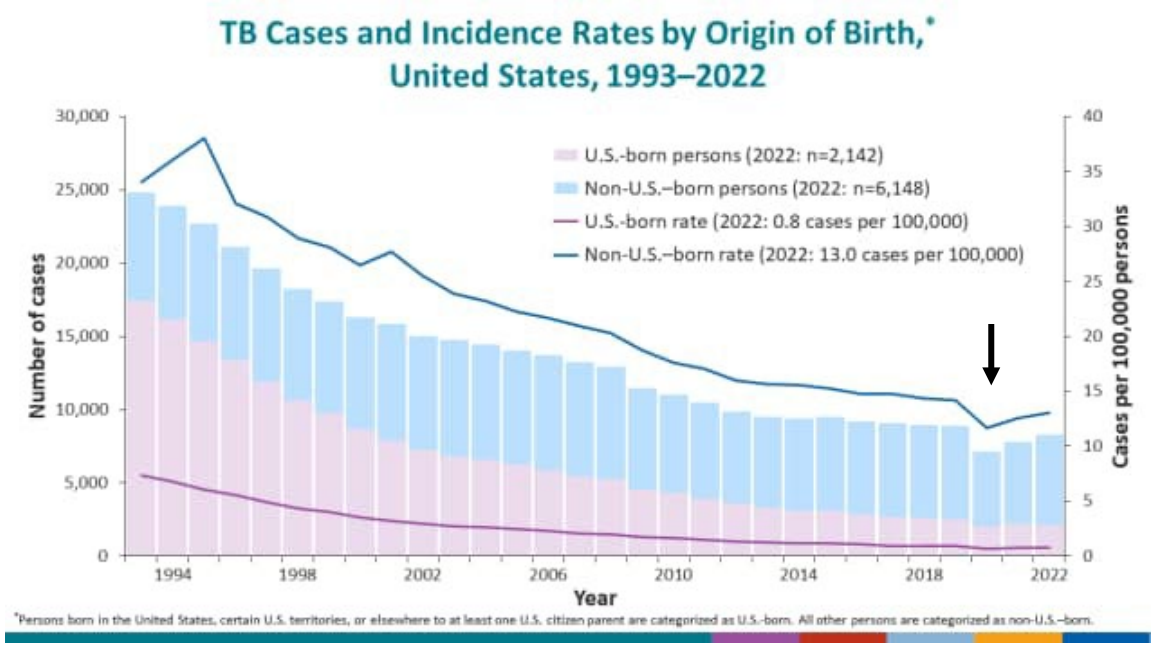
	MILESTONES		TARGETS	
			SDG*	END TB
	2020	2025	2030	2035
Reduction in number of TB deaths compared with 2015 (%)	35%	75%	90%	95%
Reduction in TB incidence rate compared with 2015 (%)	20%	50%	80%	90%

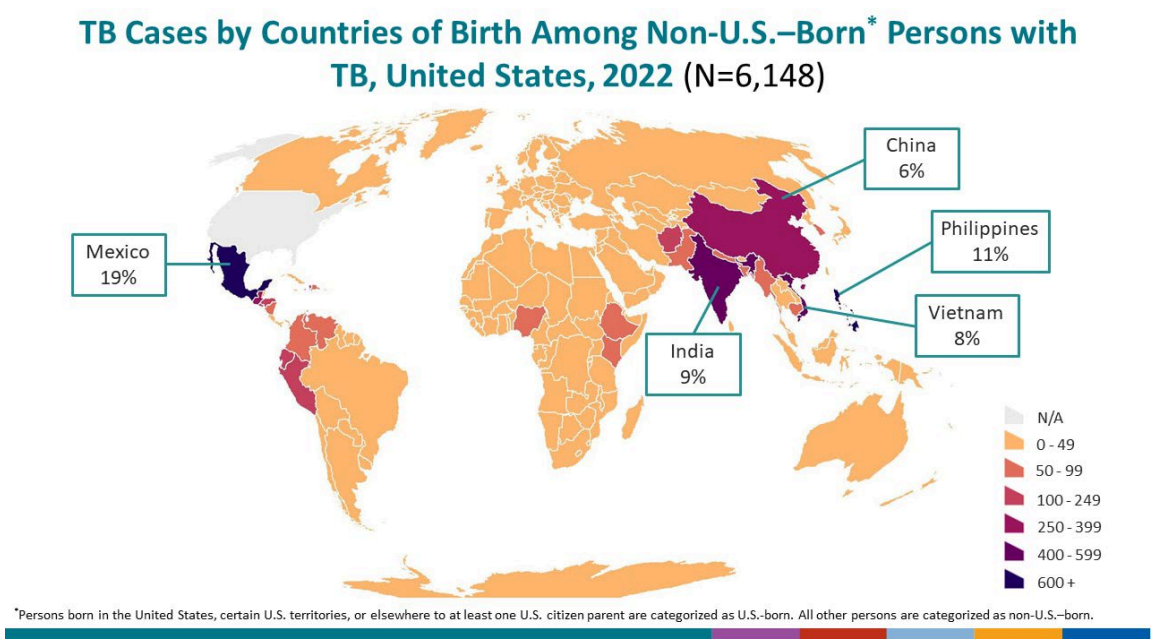
Diagnosis:

- Access to rapid & accurate diagnostics
  - Target >90% of cases
  - Used in only 47% of cases in 2022
- Universal access to DST
  - Target 100%
  - 2022 WHO Report – RIF, 73%

WHO: Global Tuberculosis Report 2023

TB in the US

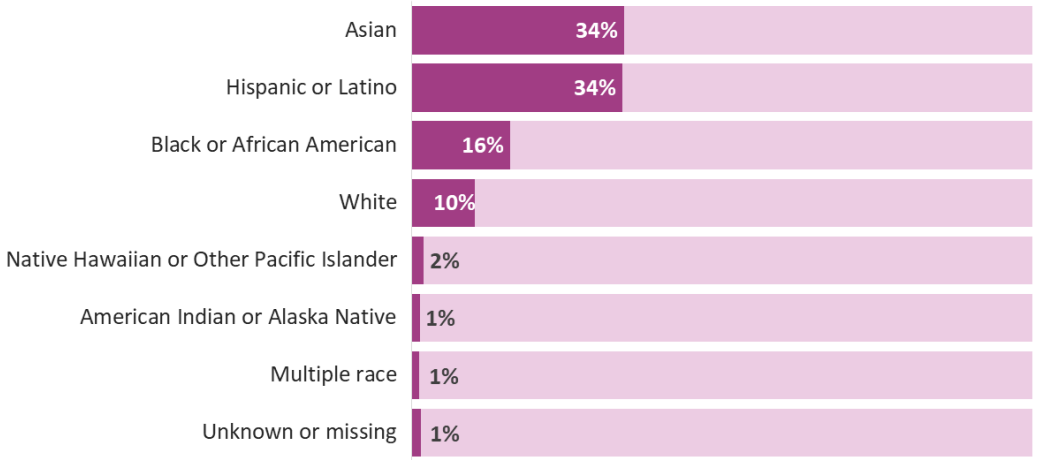




In 2022, 74% of new cases in the US were non-US born

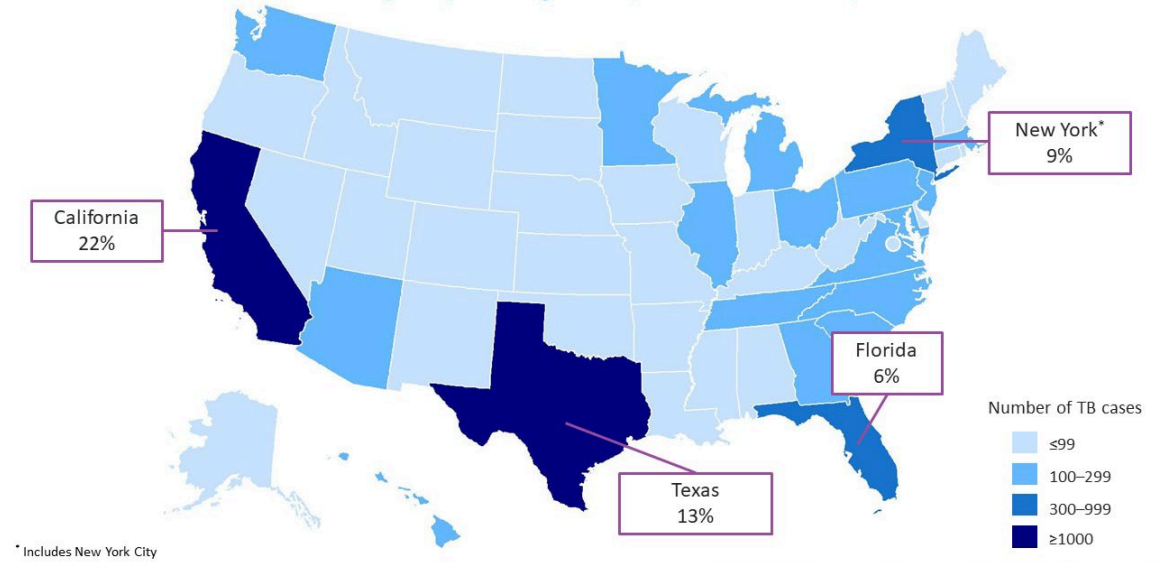
CDC

Percentage of TB Cases by Race/Ethnicity,* United States, 2022 (N=8,331)



*Persons who identified as Hispanic or Latino were categorized as "Hispanic," regardless of self-reported race. Persons who did not identify as Hispanic or Latino were categorized by self-reported race; if more than one race was reported, the person was categorized as "Multiple race."

TB Cases by Reporting Area, United States, 2022



* Includes New York City

CDC

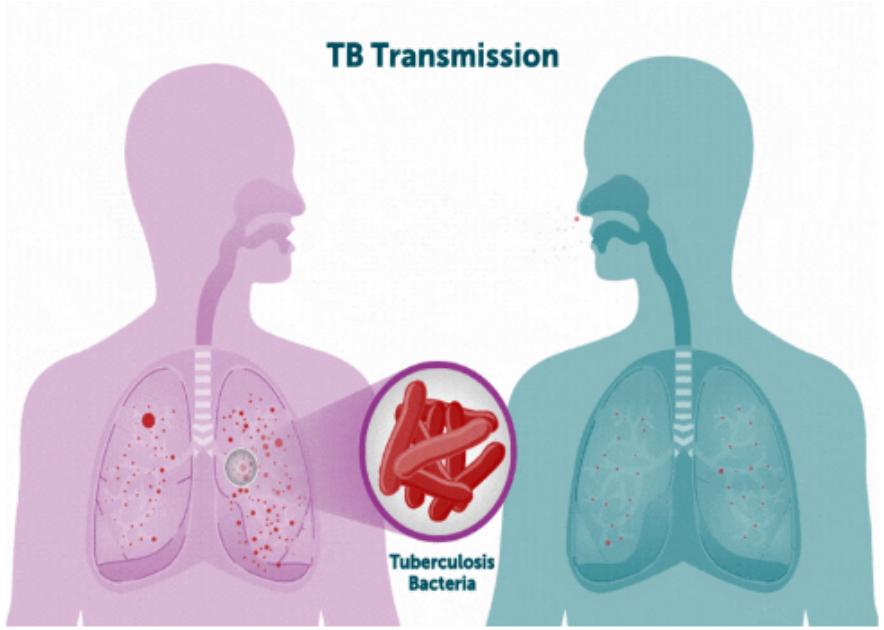
TB Transmission

TB is spread through the air from person to person. Tiny particles containing *M. tuberculosis* (TB bacteria) may be expelled into the air when a person with infectious TB of the lungs, airway, or larynx:

- Coughs
- Speaks
- Sings

People nearby may breathe in these TB bacteria (illustrated by the person on the right) and become infected.

These particles, called airborne **droplet nuclei**, can remain in the air for several hours, depending on the environment.



The dots in the air represent airborne droplet nuclei spreading through the air.

TB is **NOT** spread by:

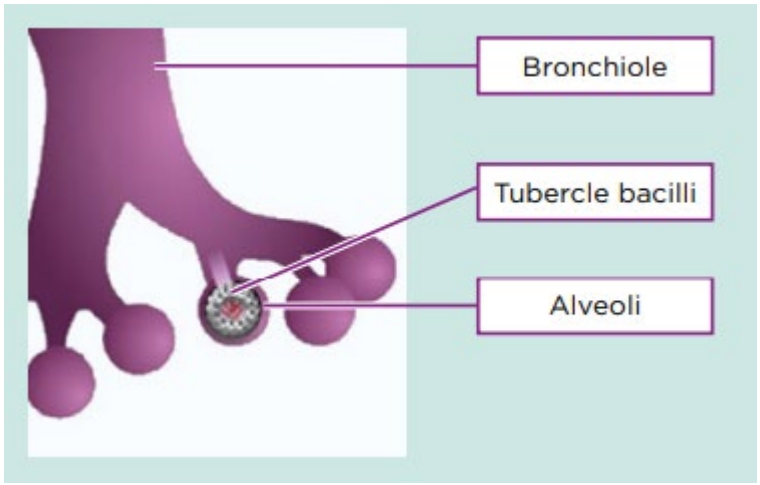
Sharing toothbrushes

Saliva from kissing

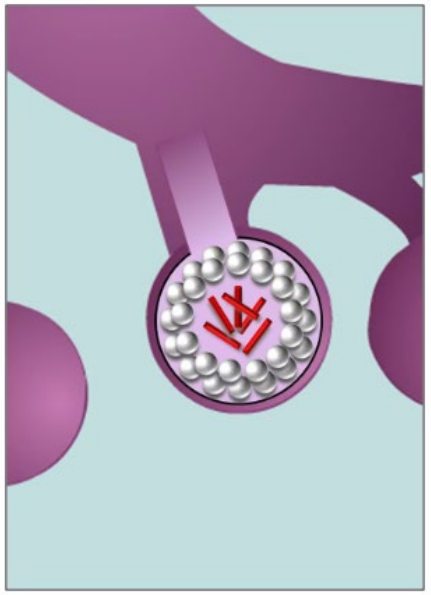
Shaking someone's hand

Touching bed linens or toilets

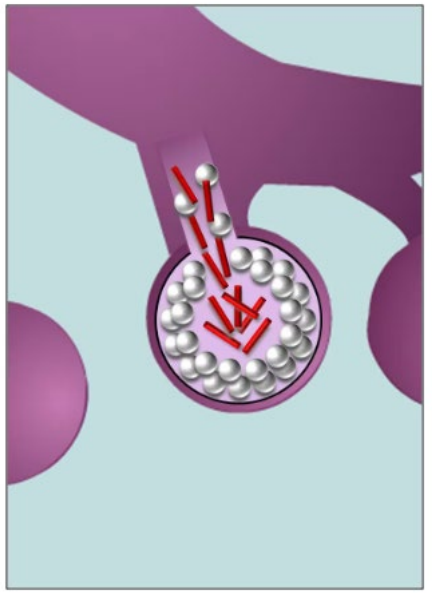
Sharing food, drink, or utensils



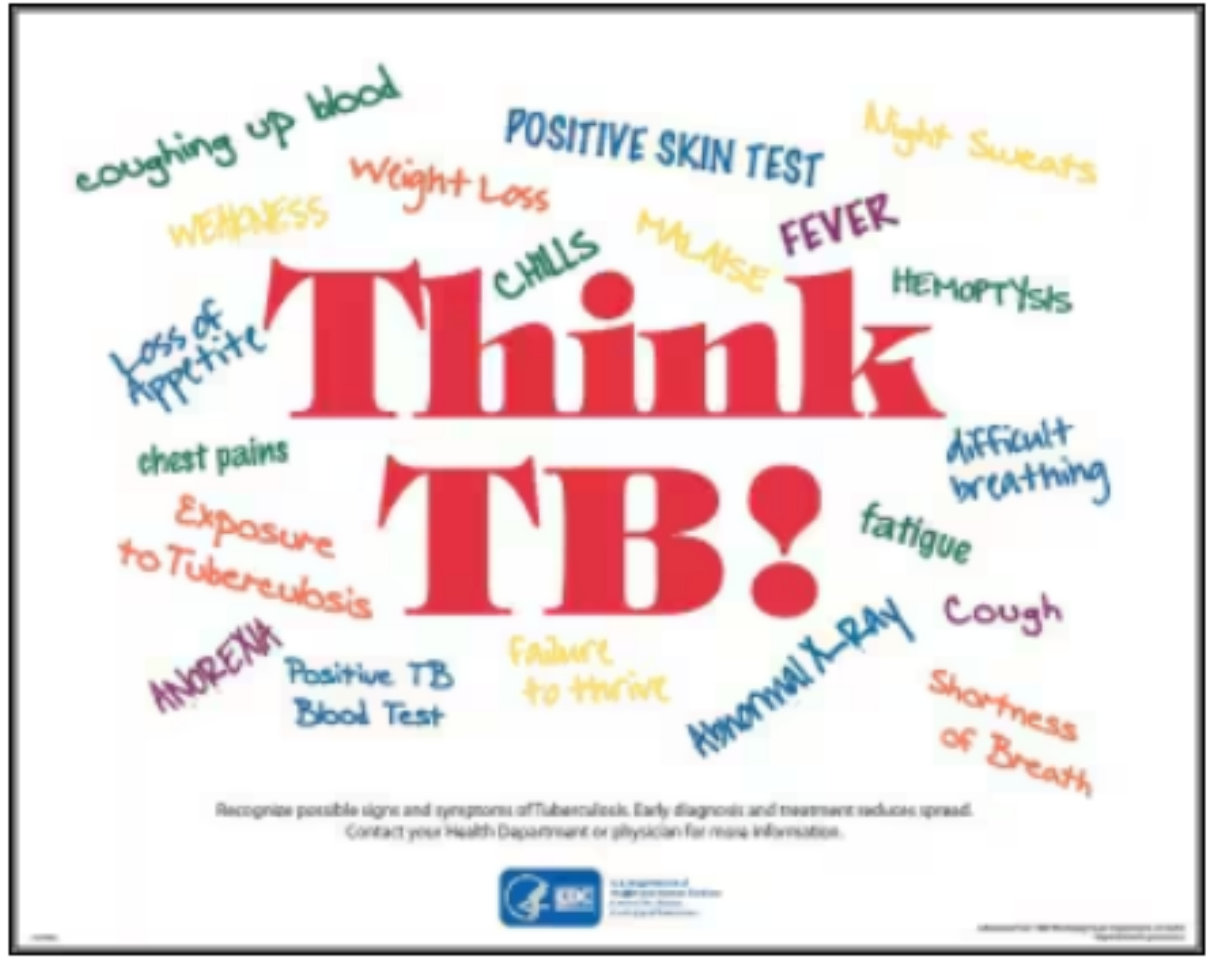
Latent TB



Active TB



Risk Factor	Risk of Developing TB Disease	Description
TB infection and no risk factors	About 10% over a lifetime	For people with TB infection, **no risk factors**, and no treatment, the risk is about 5% in the first 2 years after infection and about 10% over a lifetime.
TB infection and diabetes	About 30% over a lifetime	For people with TB infection, **diabetes**, and no LTBI treatment, the risk is about 30% over a lifetime (3 times as high as those with no risk factors).
TB infection and HIV infection	About 7% to 10% PER YEAR	For people with TB infection, **untreated HIV infection** and with no LTBI treatment, the risk is about 7% to 10% PER YEAR, a very high risk over a lifetime.



The key to diagnosing TB is for clinicians to "think TB" when they see a patient with signs and symptoms of TB disease.

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

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

MAYO CLINIC

Center for Tuberculosis

Interferon gamma release assays (IGRA)

-

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

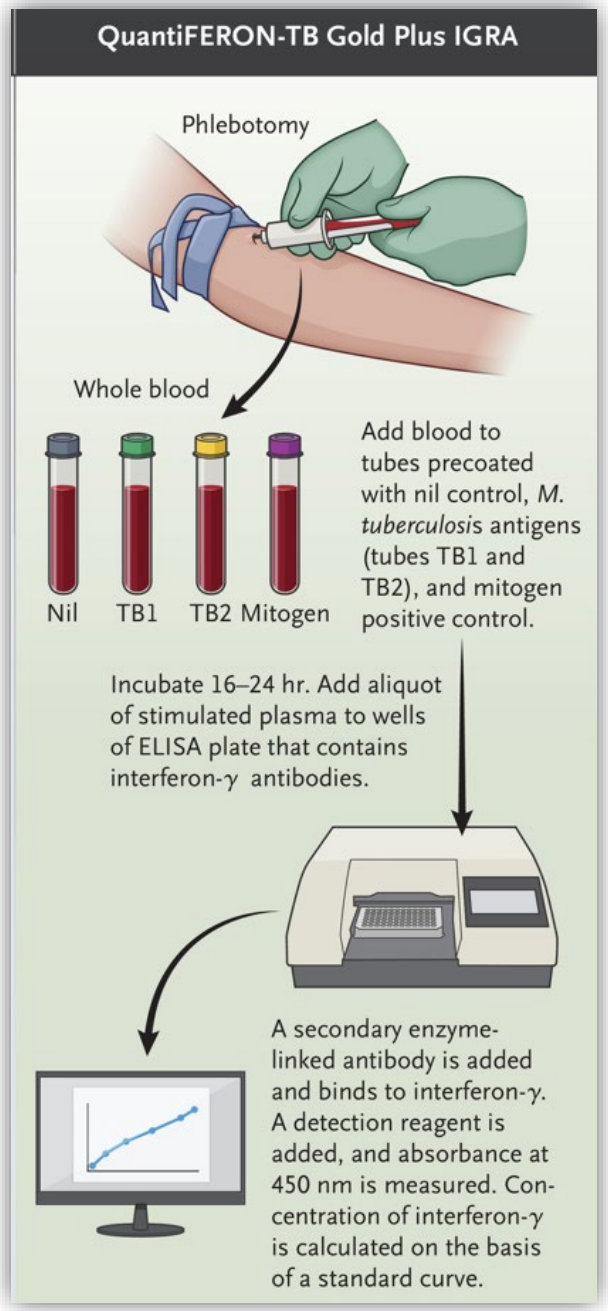
T.SPOT ®-TB (Oxford Immunotec)

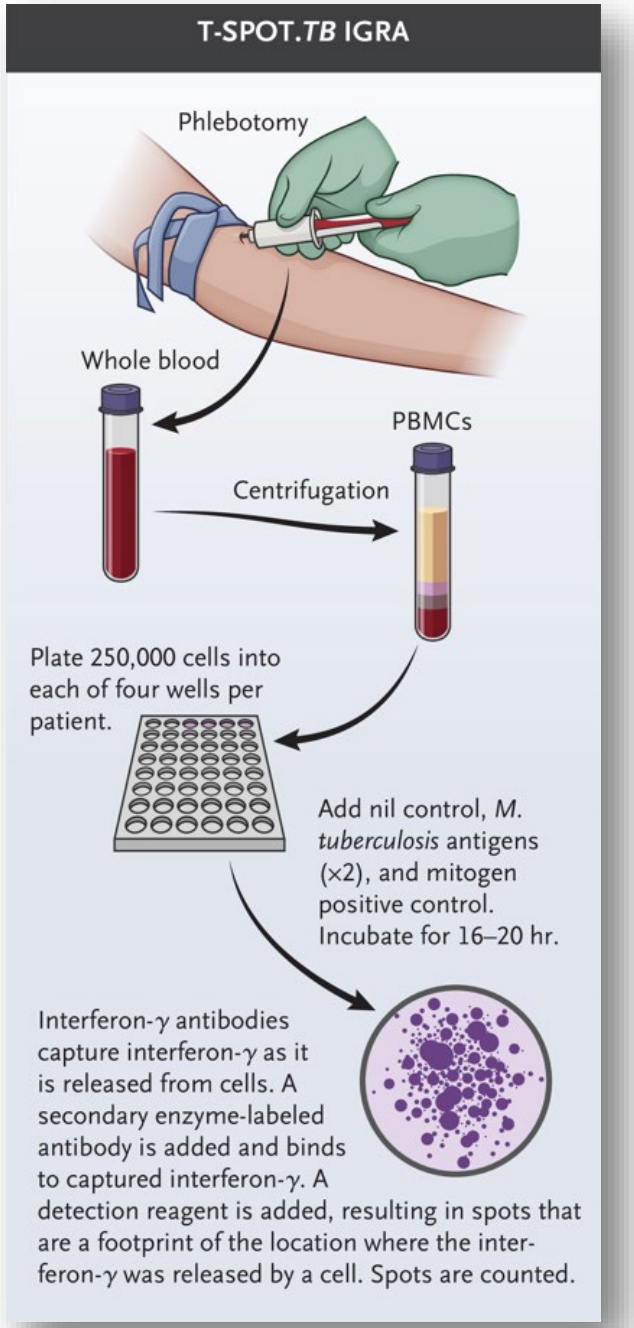
IGRAs are **indirect** tests for infection with MTB

Patients infected with MTb have primed T-cells

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

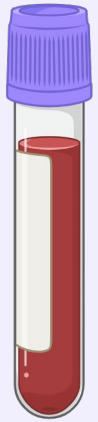
IFN-γ detected by EIA/CIA or ELISPOT methods


QuantiFERON-TB Gold Plus IGRA
Phlebotomy
Whole blood
Nil
TB1
TB2
Mitogen
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.


T-SPOT.TB IGRA
Phlebotomy
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.

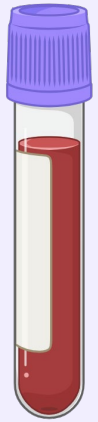
CDC

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-

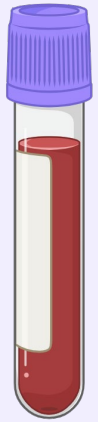
slide courtesy of M. Fida, MD

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-

slide courtesy of M. Fida, MD

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

slide courtesy of M. Fida, MD

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

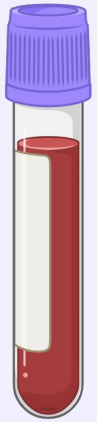
Immunosuppressed patients
Technical issues

What to do?

Consider getting T spot test

slide courtesy of M. Fida, MD

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

slide courtesy of M. Fida, MD

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

Variability for the QFT Assays

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

With-in subject variability: ± 0.60 IU/mL

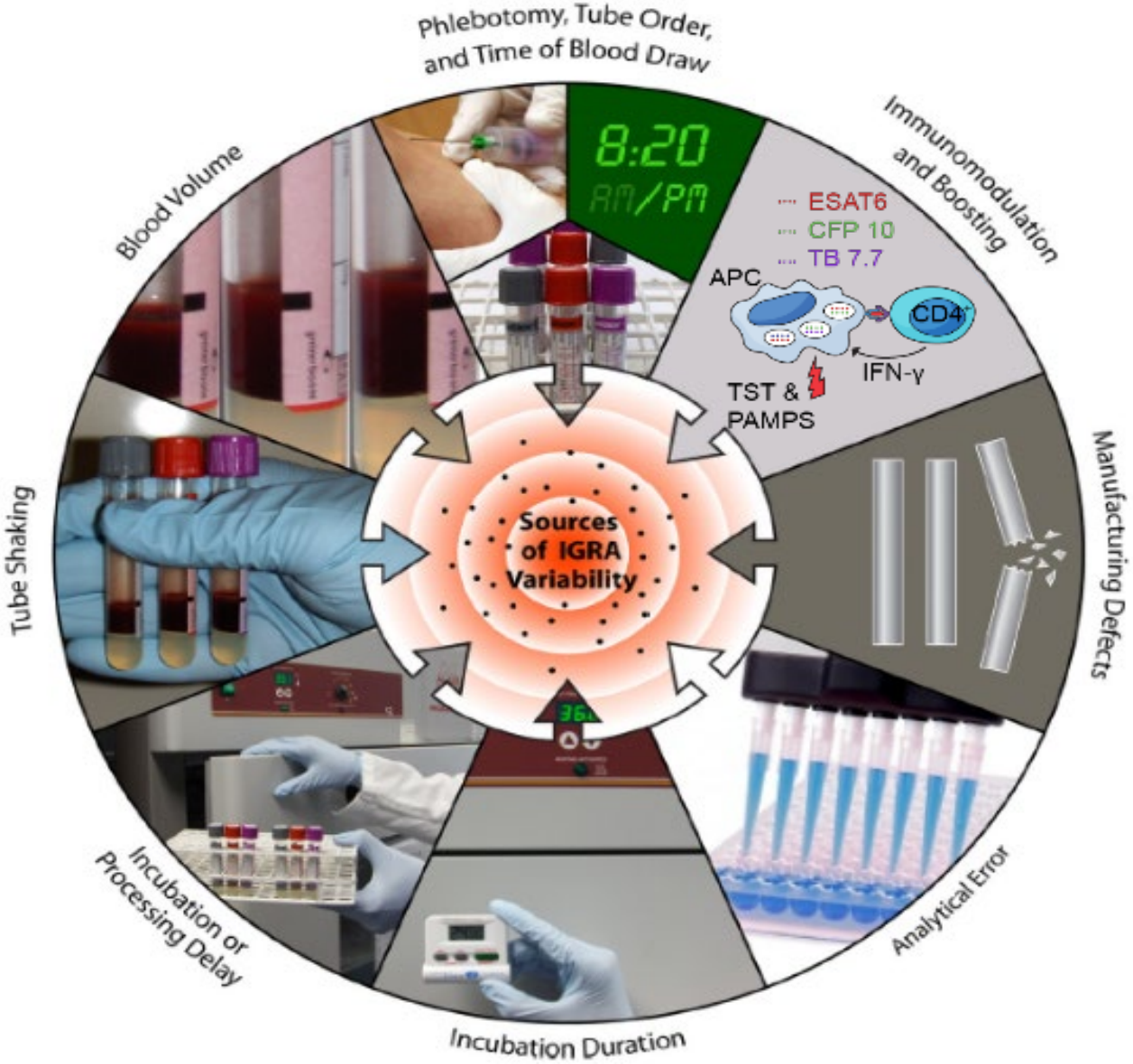
Interpret positive QFT values between

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

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)

MAYO CLINIC

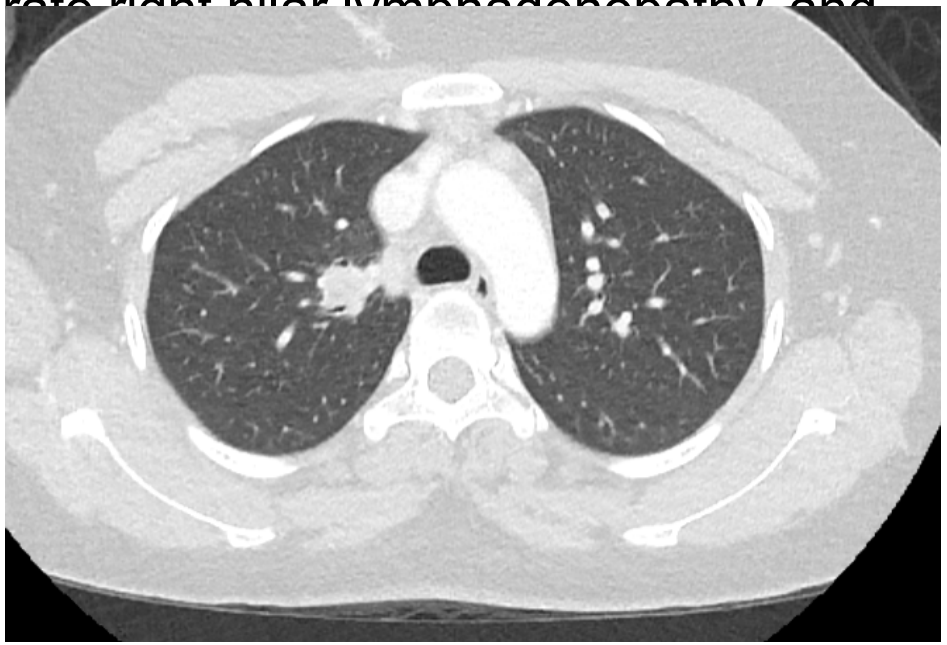
Center for Tuberculosis

Bacteriological examination

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.

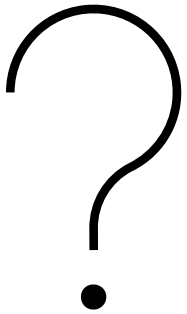


No previous QuantiFERON, Current QuantiFERON is positive

What is the best next step?

Clinical case

Polling Question

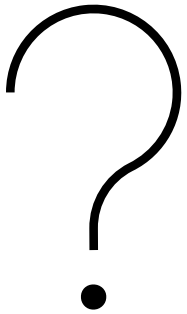


Given the previous history of TB with incomplete therapy, underlying suspicion for TB was high.

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



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

- The specimens should be examined and cultured in a laboratory that specializes in testing for M. tuberculosis.

- Optimal bacteriologic examination has **5** parts:

    1. Specimen collection, transport, and processing

    2. AFB smear classification

    3. Direct detection is in clinical specimens using nucleic acid amplification tests (NAAT) and, as applicable, molecular detection of resistance

    4. Specimen culture and identification of M. tuberculosis

    5. Drug susceptibility testing using growth-based and molecular methods (phenotypic and genotypic)

Patient coughing up sputum

Laboratory Safety for Mycobacteria

M. tuberculosis / M. bovis are BSL III pathogens when growing in culture

- respiratory protection
- work performed in BSC
- negative pressure room, directional HEPA-filtered exhaust
- solid-front gowns, gloves, etc
- tuberculocidal disinfectants

slide courtesy of N. Wengenack, PhD

- At least **3** consecutive sputum specimens are needed, each collected in 8- to 24-hour intervals, with at least one being an early morning specimen.

- If possible, specimens should be obtained in an airborne infection isolation room

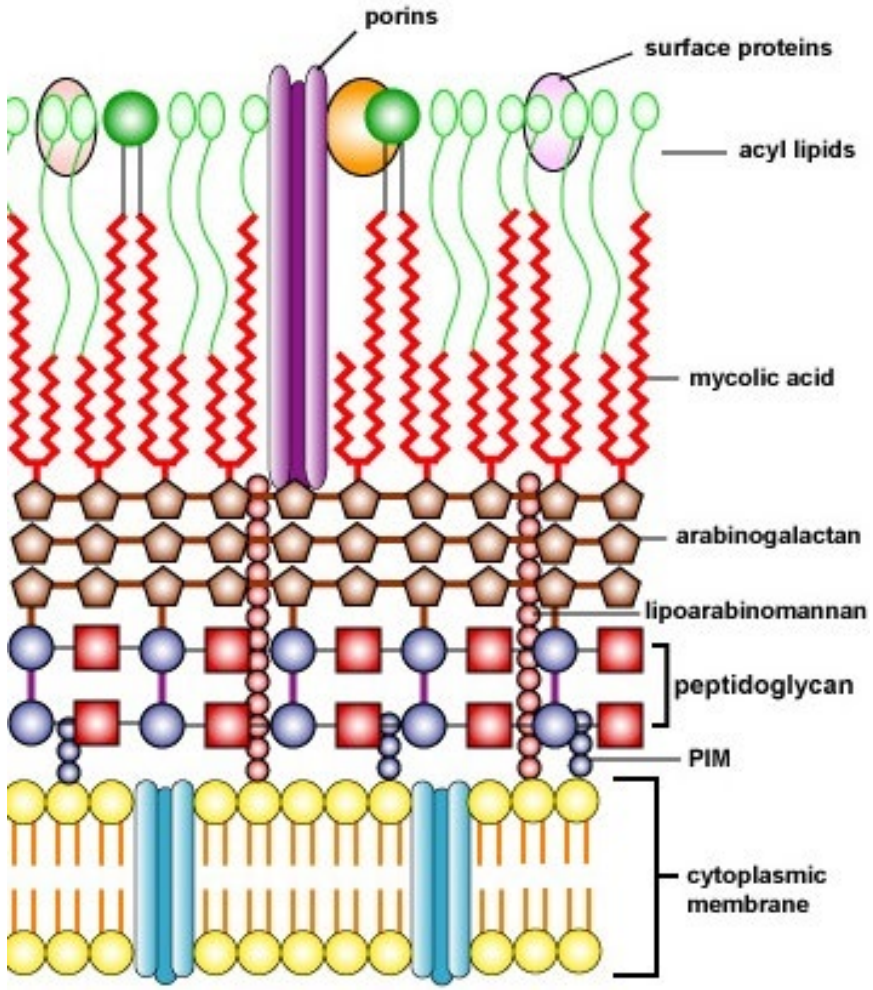
- Other acceptable specimens: induced sputum, bronchoalveolar lavage, gastric aspirate

Patient in a sputum collection booth

Mycobacterium tuberculosis complex
General Characteristics

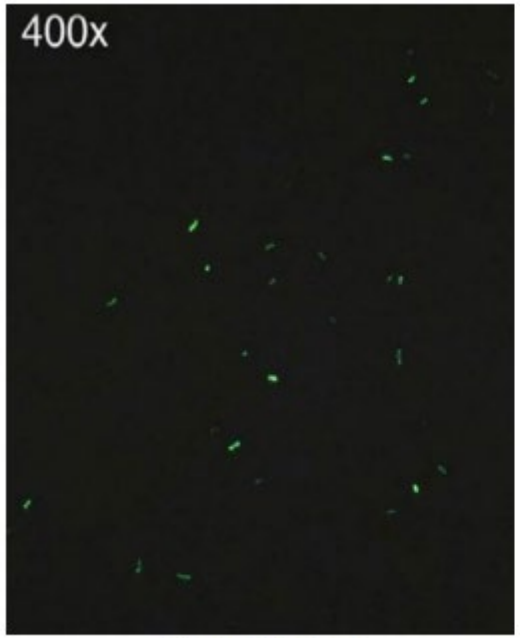
Mycobacteria, including *M. tuberculosis*, are acid-fast bacilli (AFB)

- complex cell wall with very high lipid content that functions as a permeability barrier
- very resistant to drying, desiccation, & many disinfectants and antibiotics
- use special ”acid-fast” stains for mycobacteria
  - Auramine-rhodamine (fluorescent)
  - Ziehl-Neelsen
  - Kinyoun
  - Fite (Pathology)

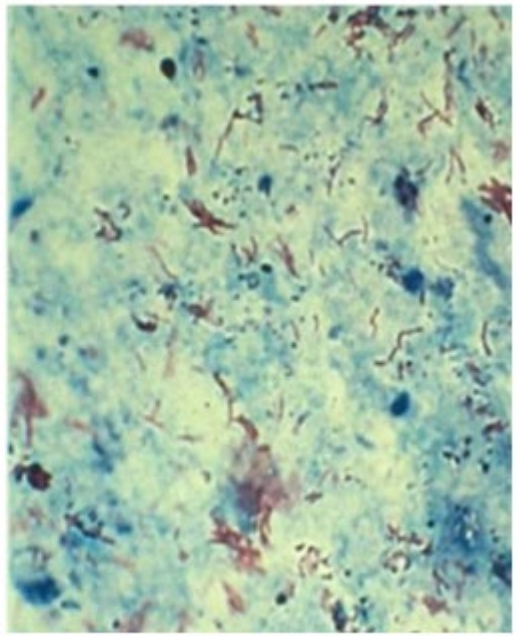


https://bio.libretexts.org/Bookshelves/Microbiology/

slide courtesy of N. Wengenack, PhD



Fluorochrome method



Ziehl-Neelsen method with tubercle bacilli shown in red

- AFB stain is relatively inexpensive but not very sensitive (need ~1,000 - 10,000 AFB/mL sputum)
- Fluorescent stains are 10% more sensitive
- Minimum sputum sample is 3 mL; optimal 5-10 mL
- Sensitivity of smear on:
  - Single sample:53%
  - Two samples: 65%
  - Three samples 70%
- Cannot distinguish M. tuberculosis from non-tuberculous mycobacteria

Fluorescent Microscopy Scale (CDC Scale)

What you see (250X)	What you see (450X)	What to report
0 AFB/ smear	0 AFB/ smear	No AFB seen
1-2/ 30 fields	1-2/ 70 fields	Report exact count; order repeat specimen
1-9/ 10 fields	2-18/ 50 fields	1+
1-9/ field	4-36/ 10 fields	2+
10-90/ field	4-36/ field	3+
>90/ field	>36/ field	4+

Table adapted from Kent, P.T. and G.P. Kubica. *Public Health Mycobacteriology; A Guide for the Level III Laboratory*. U.S. Dept. of HHS/ PHS/ CDC. 1985

Nucleic Acid Amplification tests (NAAT) or rapid molecular tests

- NAAT: amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen.
- NAAT: detect M. tuberculosis DNA in specimens in just hours, compared to a week or more for detection in culture
- CDC recommends that NAA testing be performed on at least one respiratory specimen

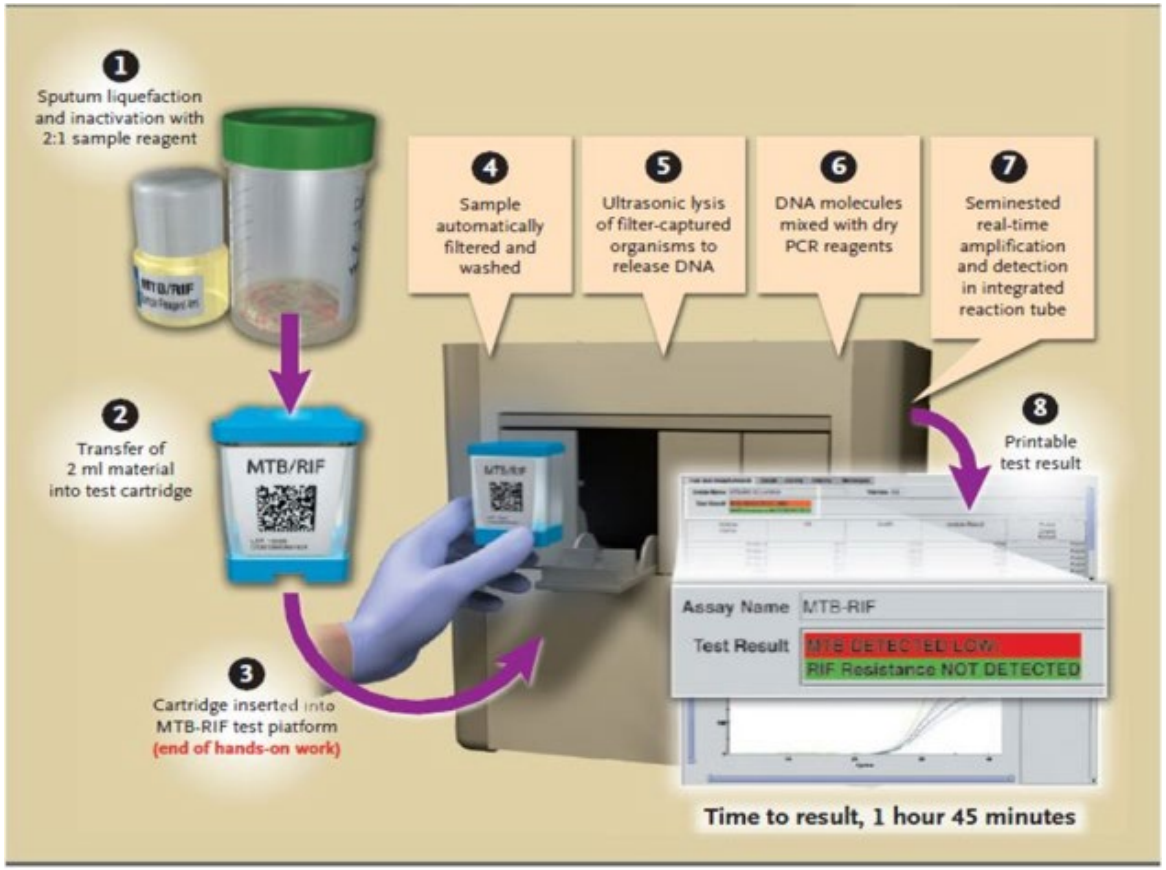
	GeneXpert® MTB/RIF	GenoType® MTBDR*plus*	HAIN GenoType® MTBDR*sl*
Company	Cepheid	HAIN Lifescience/ Bruker	HAIN Lifescience/ Bruker
Genetic loci	*rpoB* (for RIF)	*rpoB* (RIF), *katG* (INH), and *inhA* (INH)	gyrA (FQ), *rrs* (injectables), and embB (EMB)
Format	Semi-automated real-time PCR	Line probe assay	Line probe assay
FDA approved	Yes (market-authorized)	No	No

Cepheid Xpert® MTB/RIF Test

Direct detection and identification of *M. tuberculosis* from respiratory specimens

- Cepheid GeneXpert platform
- WHO-endorsed
- FDA-approved for respiratory specimens
- off-label use reported for CSF and BAL specimens
- detects *M. tuberculosis* complex and provides information about RIF resistance
- results in about 2 hrs; minimal hands-on needed
- Sensitivity 99% for AFB smear positive and 67% for smear negative

- additional tests outside the U.S,
  - Xpert MTB/RIF Ultra – enhanced sensitivity
  - Xpert MTB/XDR – additional AST cartridge for 9 target genes (INH, fluoroquinolones, injectibles (amikacin, kanamycin, capreomycin) and ETH)



Source: www.finddiagnostics.org

Behme (2010) N Engl J Med. 363:1005-15; slide adapted from N. Wengenack, PhD

Benefits of using NAAT Direct Detection of M. tuberculosis in Clinical Specimens include:

- Earlier laboratory confirmation of TB disease.
- Earlier treatment initiation.
- Improved patient outcomes.
- Interruption of transmission by early diagnosis, respiratory isolation, and appropriate treatment.
- Earlier, more efficient use of respiratory isolation (i.e., informed decisions about starting and stopping respiratory isolation).
- Earlier initiation of contact investigation.
- More effective public health interventions.
- Earlier detection of drug resistance when certain NAAs are used (e.g., Gene Xpert)

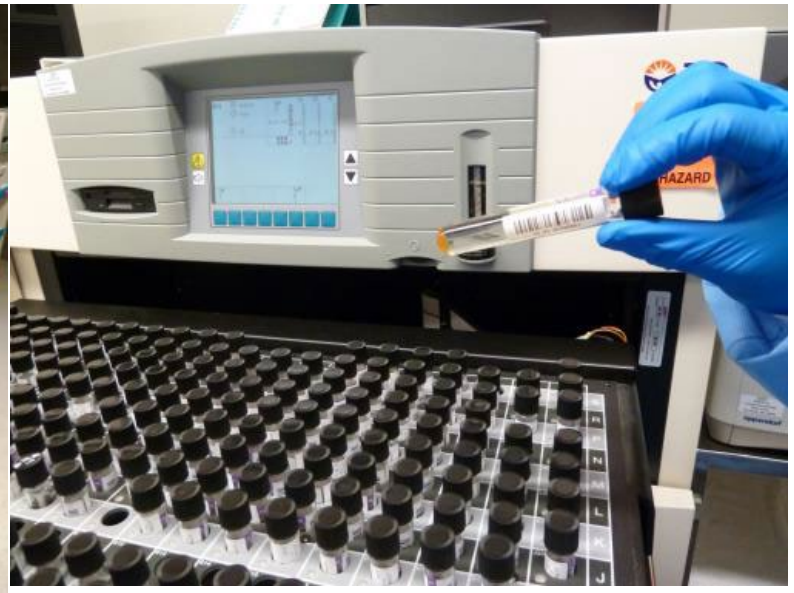
Mycobacterial Culture

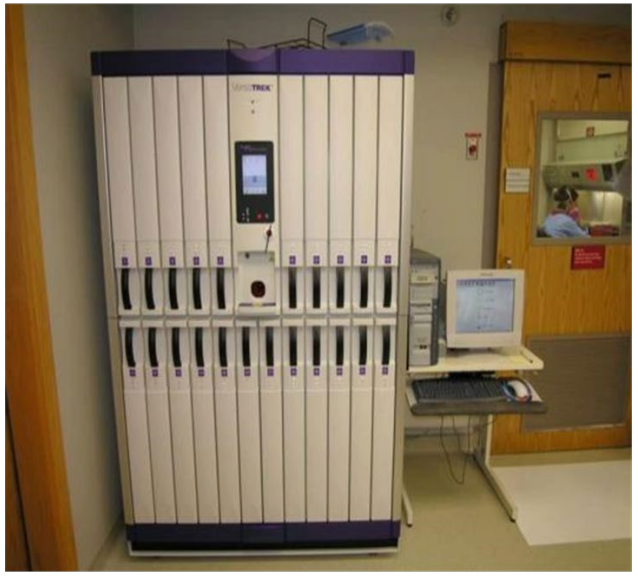
Sensitivity of culture is better than smear (only need 10-100 AFB/mL of specimen)

Culture Media

- solid medium - Lowenstein-Jensen (LJ) egg-based or Middlebrook enriched agar
- broth/liquid medium - FDA-cleared systems for non-blood specimens are the BACTEC MGIT (BD) and VersaTREK (TF)
- mycobacteria grow faster in broth but there are some strains that prefer solid medium
- utilize PANTA (antibacterials/antifungal) to suppress other bacteria and fungi and OADC enrichment to promote growth

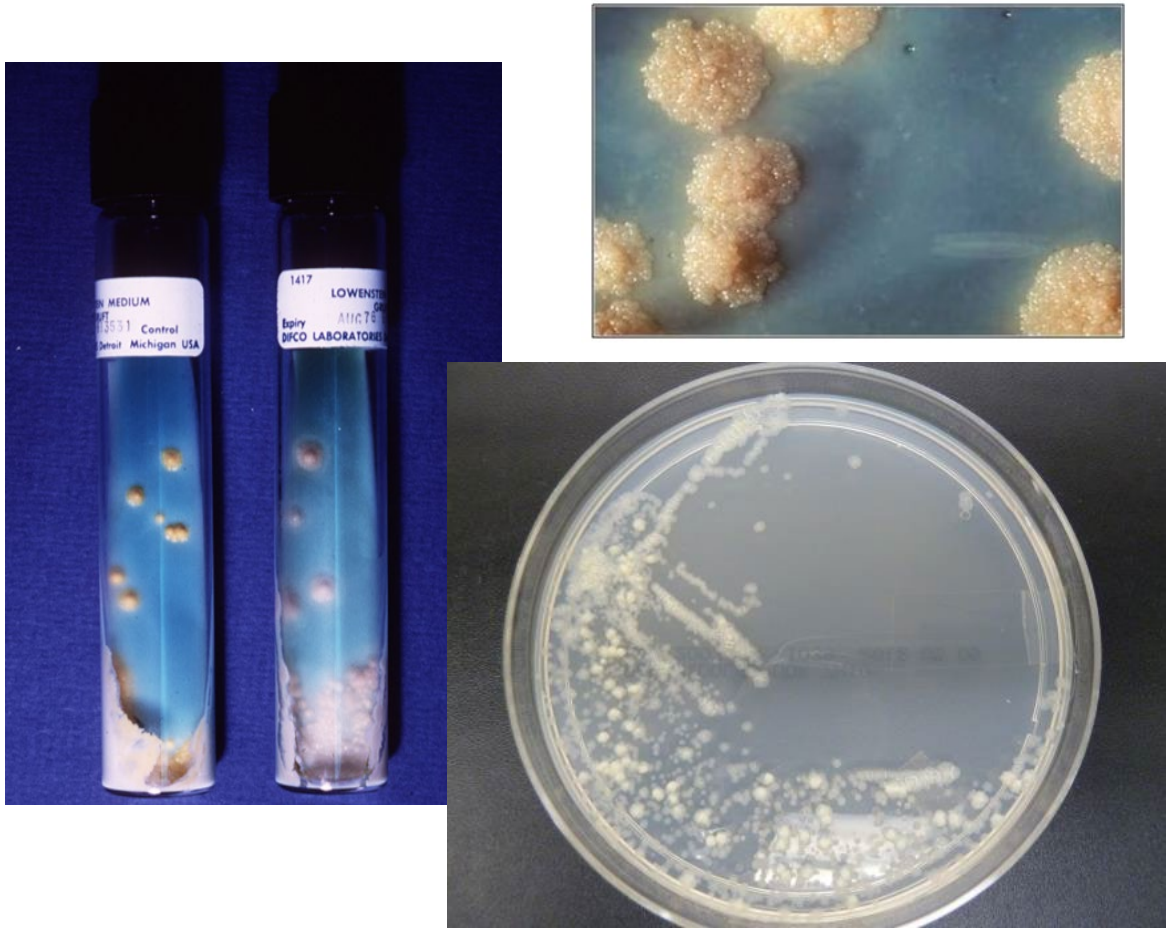
MGIT = Mycobacterial Growth Indicator Tube w/fluorescent detection





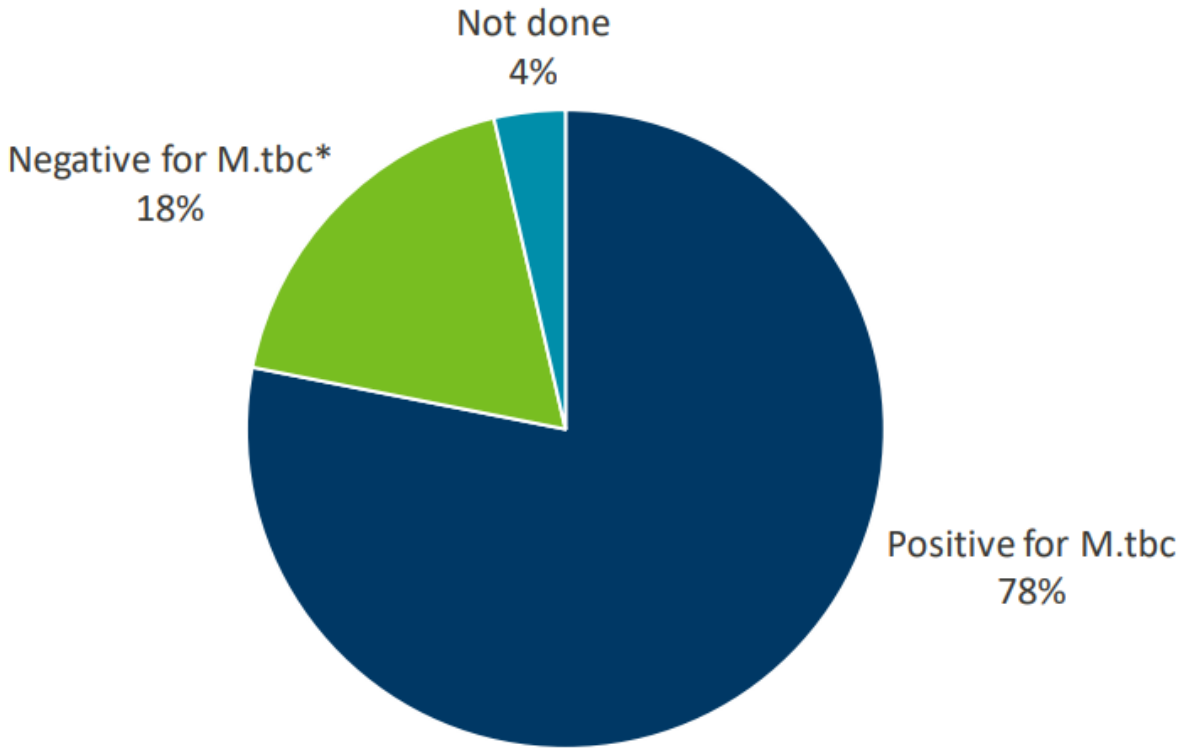
VersaTREK – headspace pressure differential indicates growth

M. tuberculosis Culture



Solid agar Mtb colony “rough and buff” morphology

Culture result for Mtb cases in MN, 2017-2021



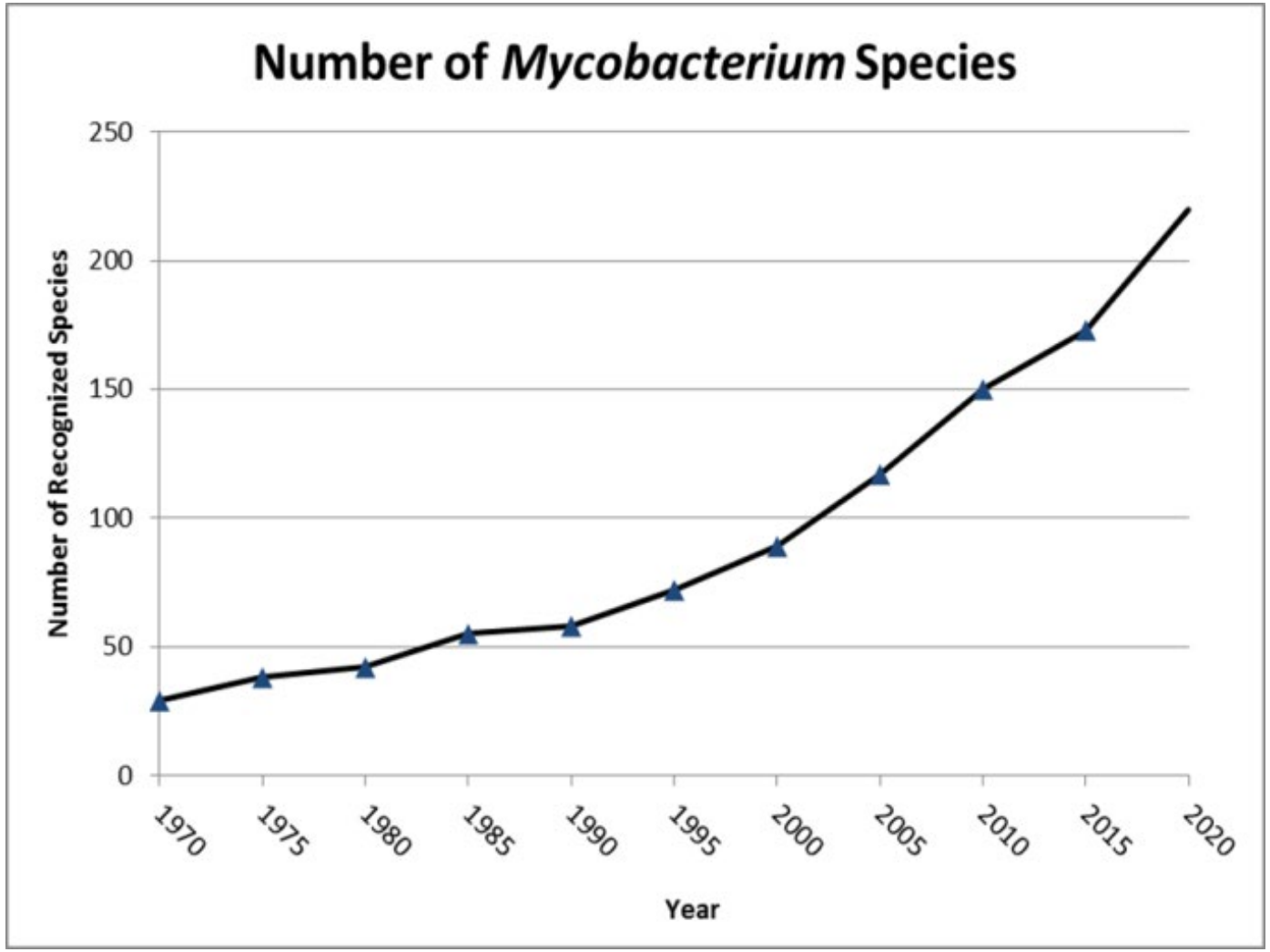
https://www.health.state.mn.us/diseases/tb/stats/tbepislides.pdf

slide adapted from N. Wengenack, PhD

Mycobacteria (2022)

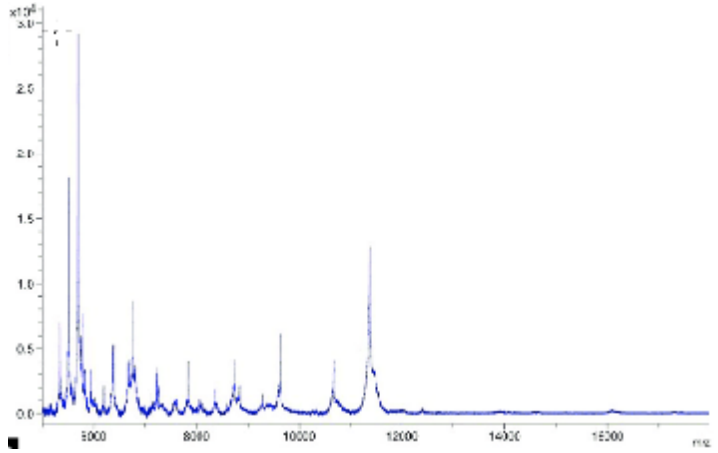
214 recognized species
22 subspecies

- *M. tuberculosis* complex
  - includes:
    - *M. tuberculosis*
    - *M. bovis* (cattle, unpasteurized milk)
    - *M. bovis* BCG (vaccine strain)
    - *M. africanum*
    - *M. microti (vole)*
    - *M. canetti*
    - *M. caprae*
    - *M. pinnepedi (seal)*
    - *M. mungi (mongoose)*

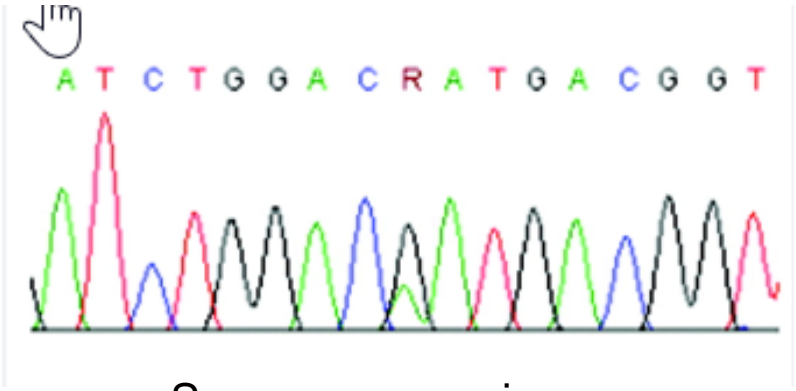


slide adapted from N. Wengenack, PhD

Identification of *M. tuberculosis* after growth in culture



- MALDI-TOF MS



Sanger sequencing

Current laboratory methods for culture identification:

- MALDI-TOF Mass Spectrometry
  - 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)

slide adapted from N. Wengenack, PhD

Differentiation of species within the MTB complex

M. tuberculosis complex species differentiation PCR available from some labs

- often uses differences in patterns with the Region of Difference repetitive sequence element (RD) in *M. tuberculosis* complex
- performed from pure culture isolate; sometimes from positive MGIT broth
- can differentiate:
  - *M. tuberculosis, M. bovis, M. bovis BCG,* other species

Expected RD signature patterns	RD1	RD4	RD9	RD12	RD9-2
M tuberculosis	+	+	+	+	+
M bovis	+	-	-	-	+
M bovis BCG	-	-	-	-	+
M africanum	+	+	-	+	+
M canettii	+	+	+	-	+
M caprae	+	+	-	-	+
M microti	-	+	-	+	+
M mungi	+	+	-	+	+
M pinnepedii	+	+	-	-	+

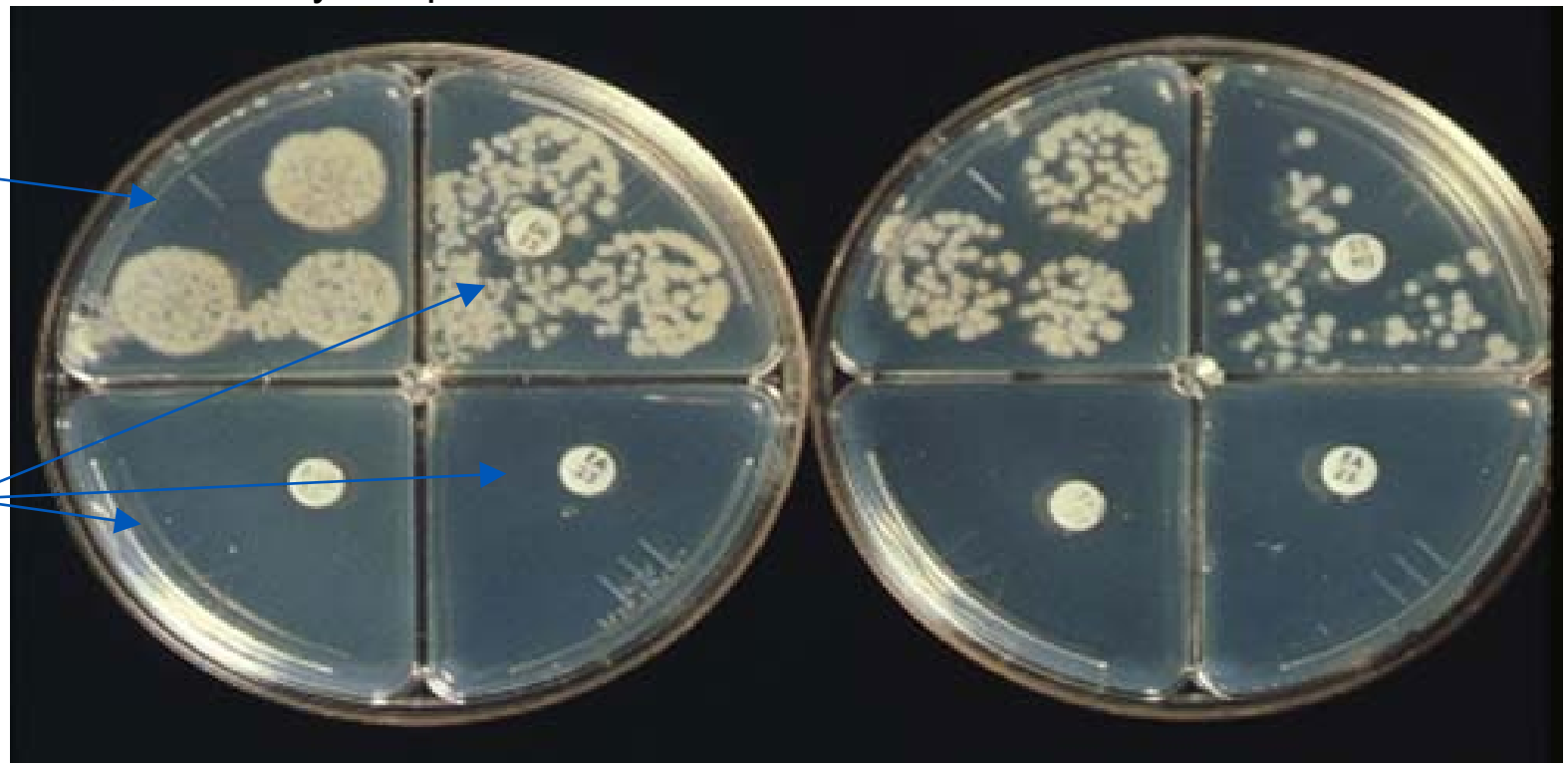
Method adapted from Halse TA, Escuyer VE, Musser KA. Evaluation of a single-tube multiplex real-time PCR for differentiation of members of the Mycobacterium tuberculosis complex in clinical specimens. J Clin Microbiol. 2011 Jul;49(7):2562-7. doi: 10.1128/JCM.00467-11. Epub 2011 May 18. PMID: 21593269; PMCID: PMC3147876.

slide courtesy of N. Wengenack, PhD

Phenotypic - 1% indirect agar proportion method *for M. tuberculosis* complex susceptibility testing

Gold standard for all drugs except pyrazinamide

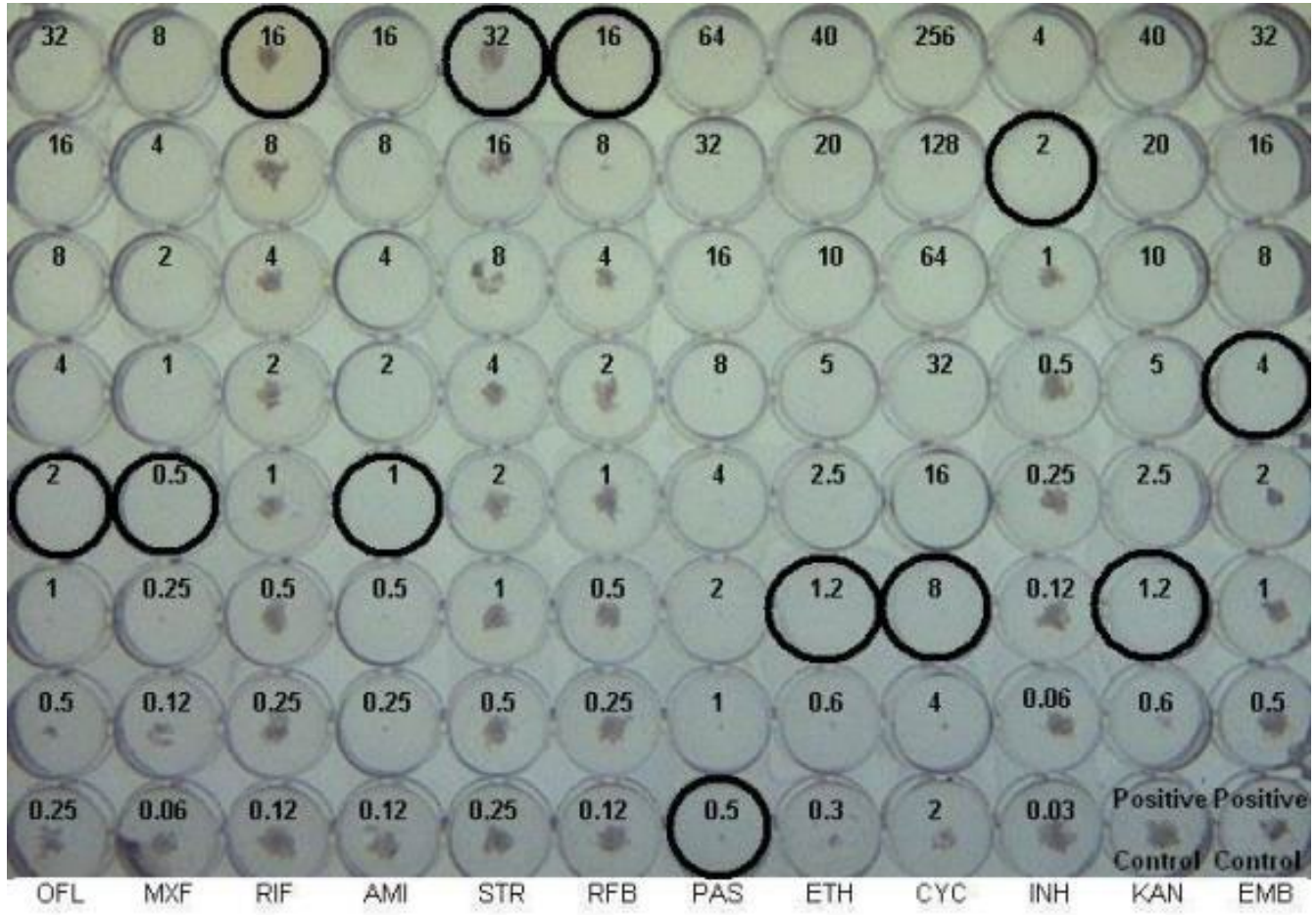
- use a single “critical concentration” of each anti-tuberculous drug
- not rapid (14-21 days)
- labor-intensive, technically complex



Organism is resistant to drug A in the upper right compartment (>1% of inoculum shown by upper left control quadrant is growing in presence of drug). Organism is susceptible to drugs B & C in the lower compartments. Control quadrant in upper left contains no drugs.

slide adapted from N. Wengenack, PhD

Phenotypic - Broth MIC testing for *Mtb* using the MYCOTB MIC Plate LDT



- Broth microdilution method
- Not rapid (14 days)
- contains INH, RIF, EMB and 9 second-line drugs
- Test 1st and 2nd line drugs simultaneously with same inoculum
- Provides MIC endpoint
- Requires skilled staff to setup and read plates

Hall L, Jude KP, Clark SL et al., Evaluation of the Sensititre MYCOTB MIC plate for the susceptibility testing of *Mycobacterium tuberculosis* complex against first and second line agents. *J Clin Microbiol*. 2012; 50:3732-4.

slide adapted from N. Wengenack, PhD

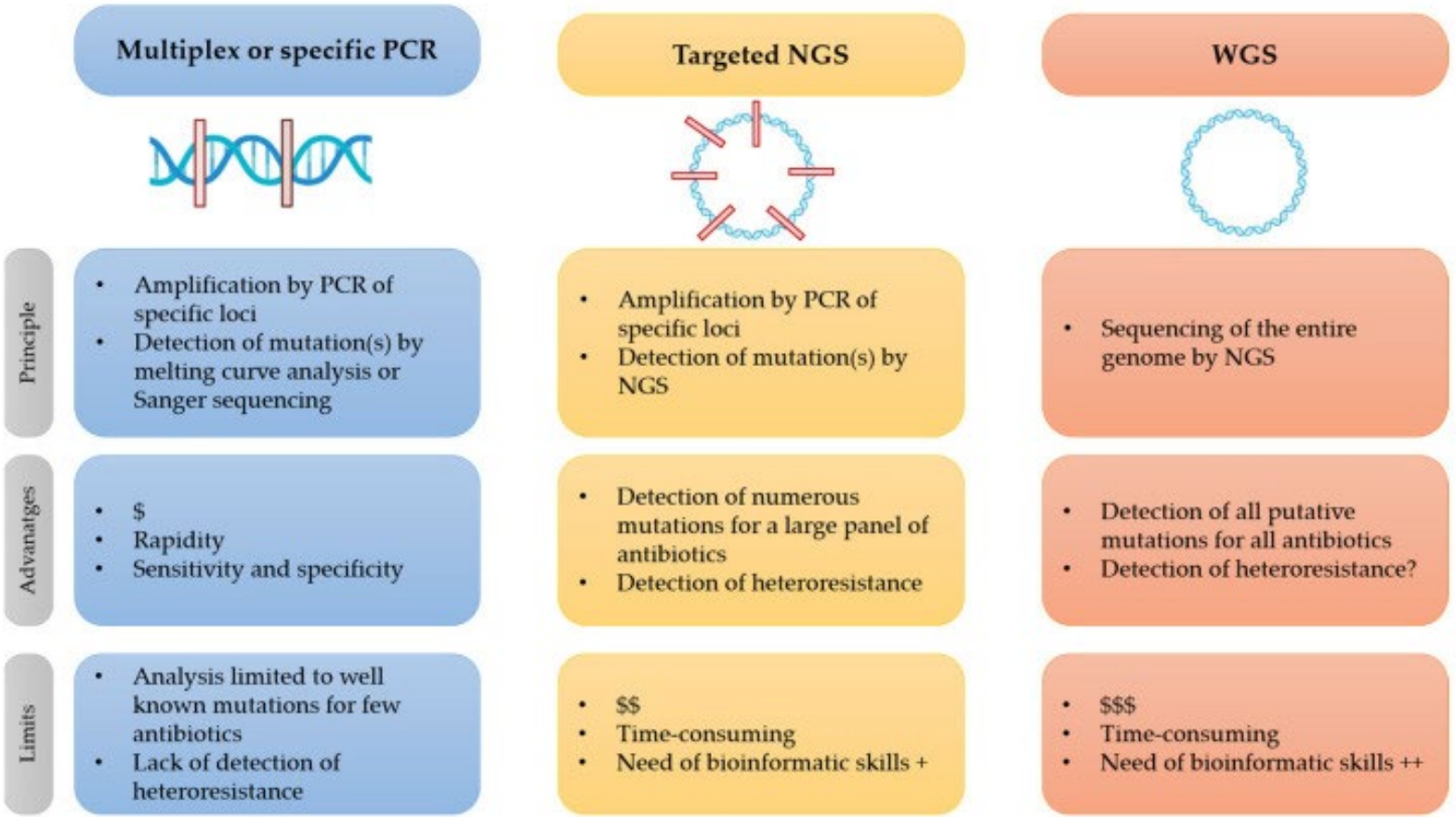
Phenotypic - "Rapid" Broth Susceptibility Testing for *M. tuberculosis* complex

FDA-cleared, semi-automated with MGIT or VersaTREK systems

- compare growth of a standard inoculum in tube +/- critical concentration of the drug
- growth at critical concentration = R
- Turn around time ~ 2 weeks

slide adapted from N. Wengenack, PhD

Most common Genotypic methods for *M. tuberculosis* complex AST



Beviere. Pathogenes. 2023 Jujl 26;12(8):978

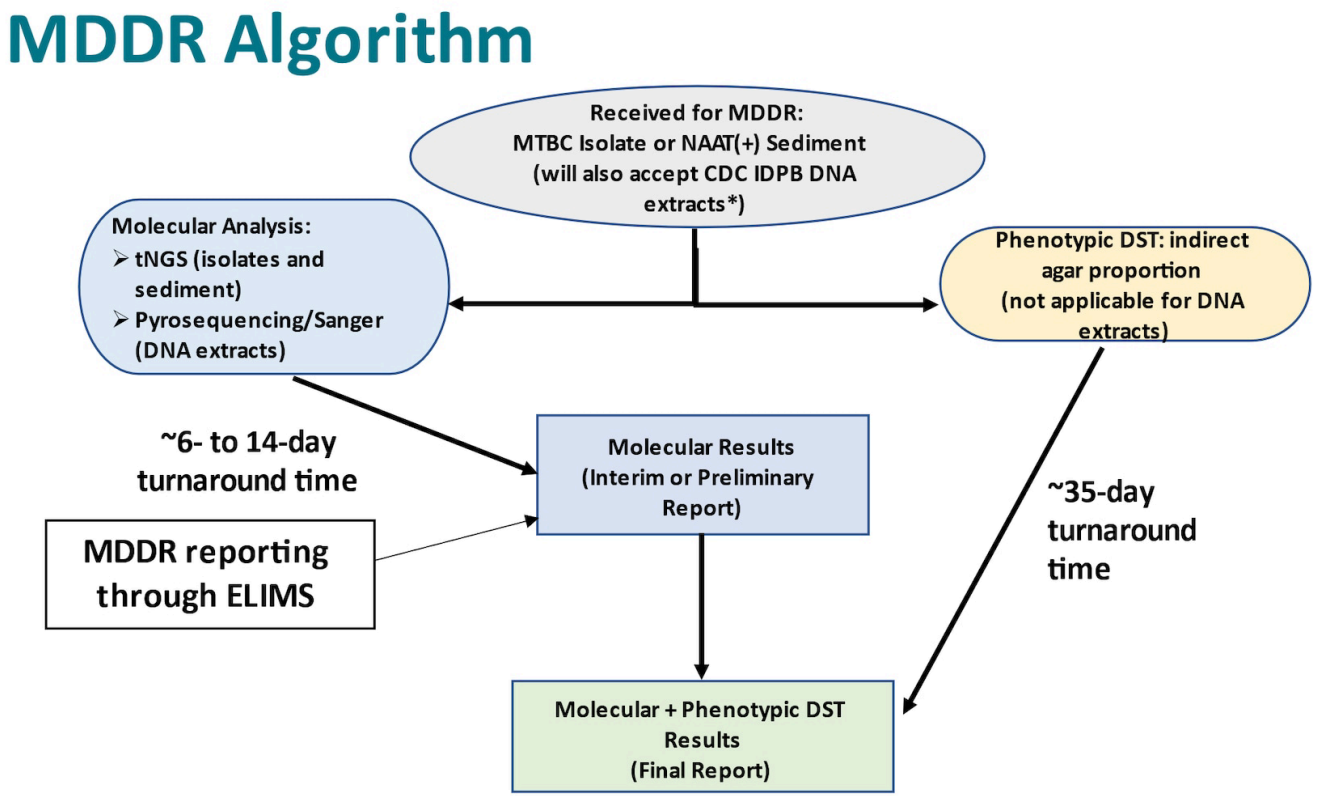
Multiplex or specific PCR:

- Available in U.S.
  - ➢Cepheid Xpert MTB/RIF – for RIF only
  - ➢LDT PCRs – e.g., MTBRP for INH only (Mayo Clinic)
  - ➢LDT Sanger sequencing – e.g., *pnc*A for PZA
- Available mainly outside of the U.S.
  - ➢Cepheid Xpert MTB/RIF Ultra – for RIF only
  - ➢Cepheid Xpert MTB/XDR – to be used in addition to MTB/RIF test; adds several gene targets for additional drugs (INH, ethionamide, fluoroquinolones, plus amikacin, kanamycin, capreomycin)

slide adapted from N. Wengenack, PhD

Targeted next generation sequencing assay (tNGS):

- Used by the MDDR service at the CDC since February 2023 (and other reference labs)
- Molecular data confirmed with phenotypic testing for majority of drugs.



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

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

Examines 24 amplicons across 16 genes providing information on more than 12 antituberculosis drugs:

- First line: RIF, INH, EMB, PZA
- Second line: FQ, AMK, CAP, KAN, BDQ, CLF, LZD.

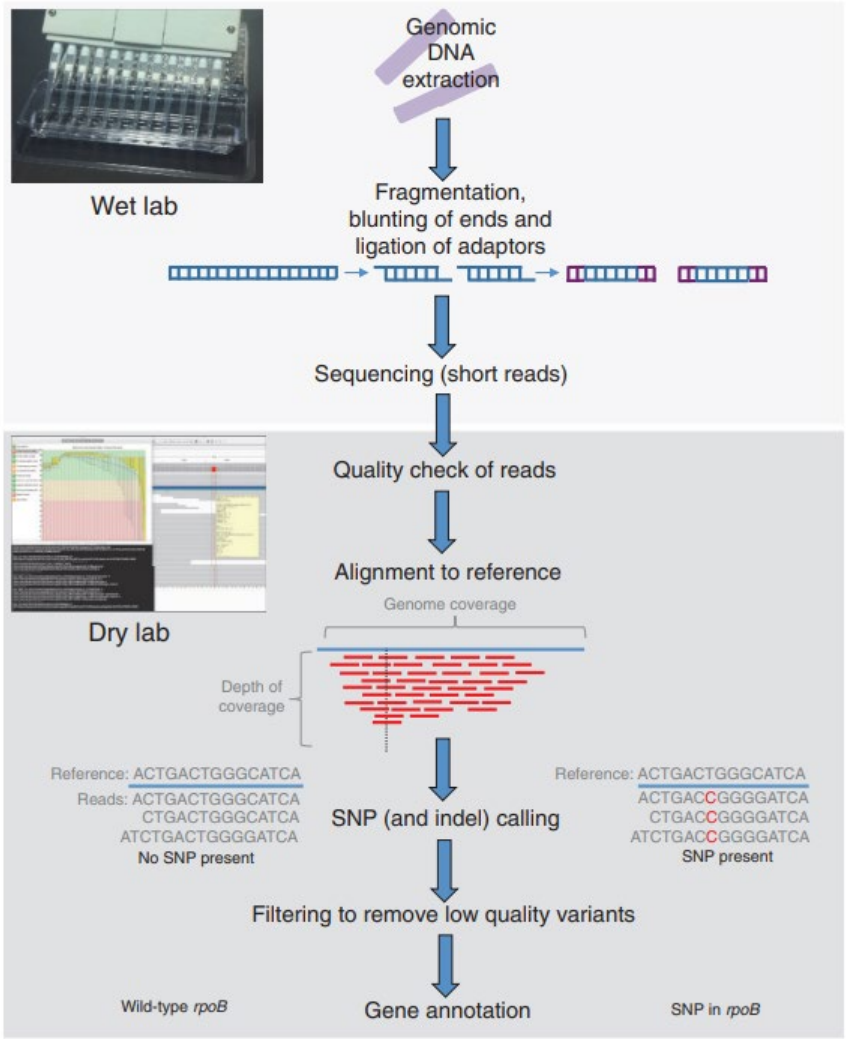
GENE TARGETS FOR GENOSCREEN DEEPLEX MYC-TB ASSAY

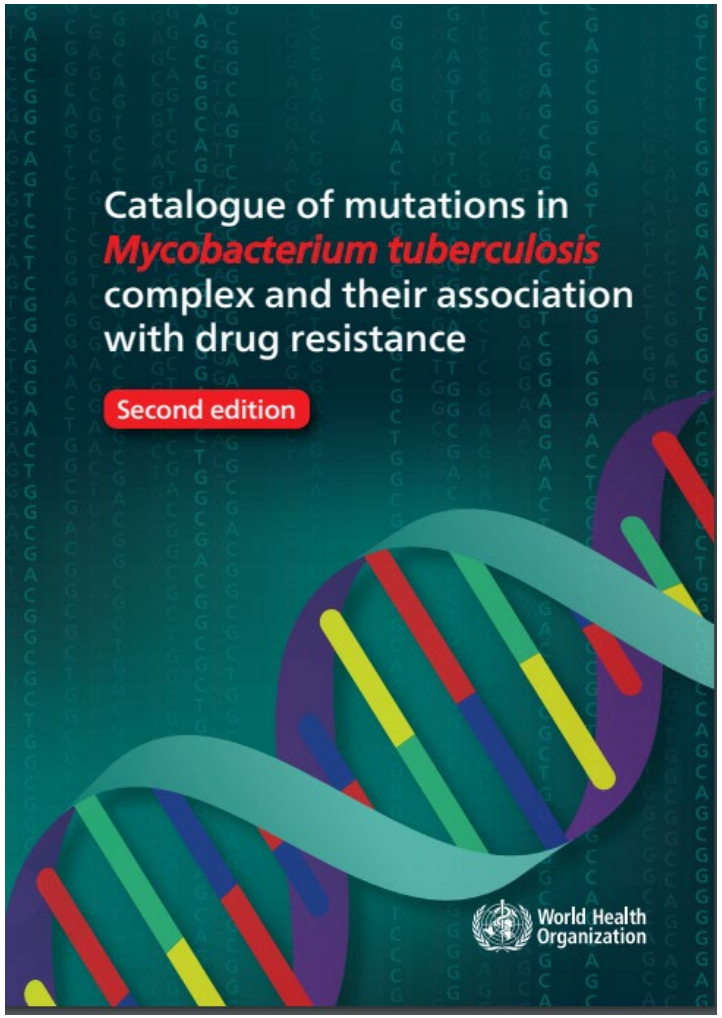
Identification of the species is performed by sequence analysis of the *hsp65* gene in combination with select other targets for closely related species

18 drug resistance markers

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

Whole Genome Sequencing (WGS):





Ther Adv Infect Dis (2016) 3(2) 4762

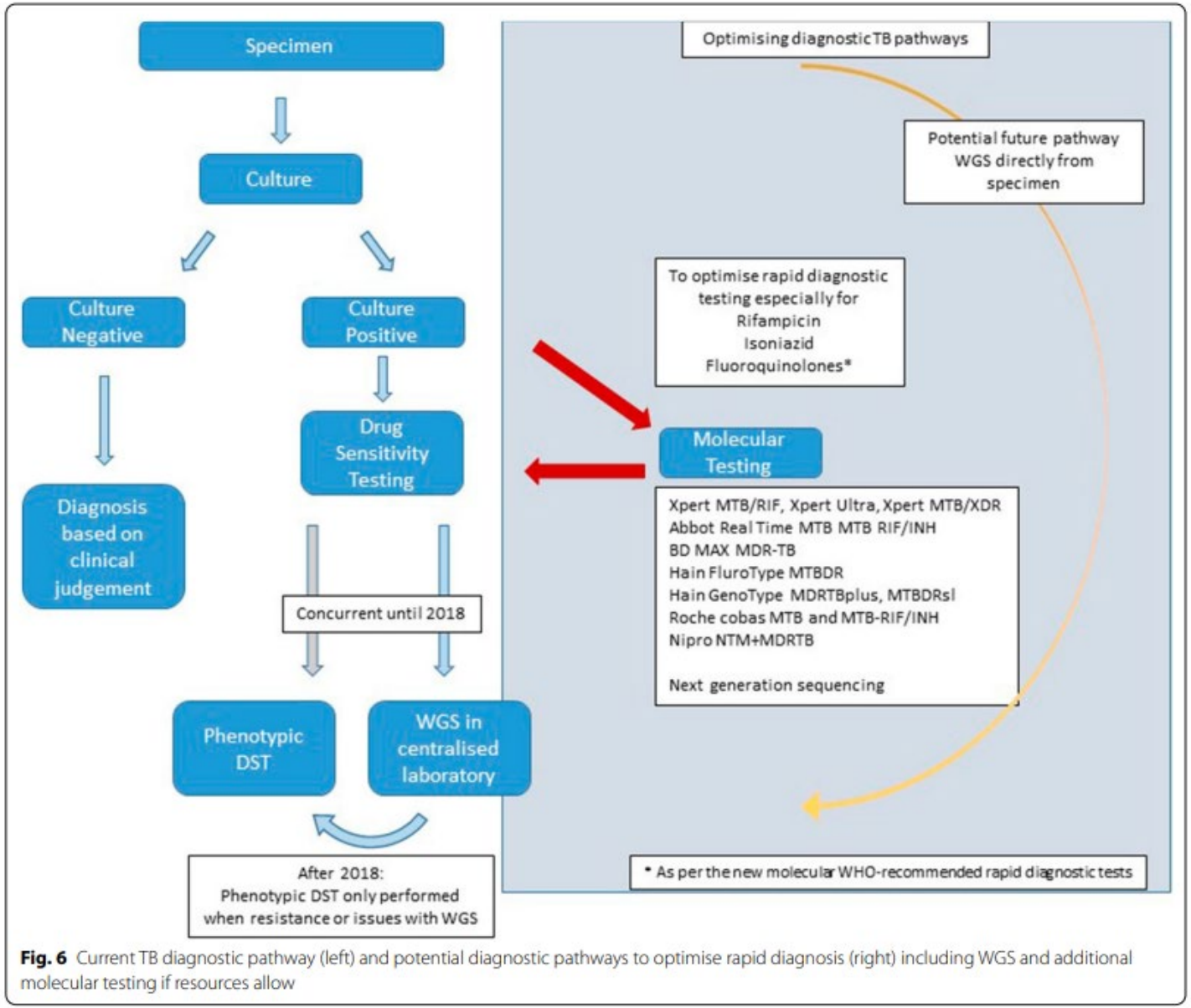
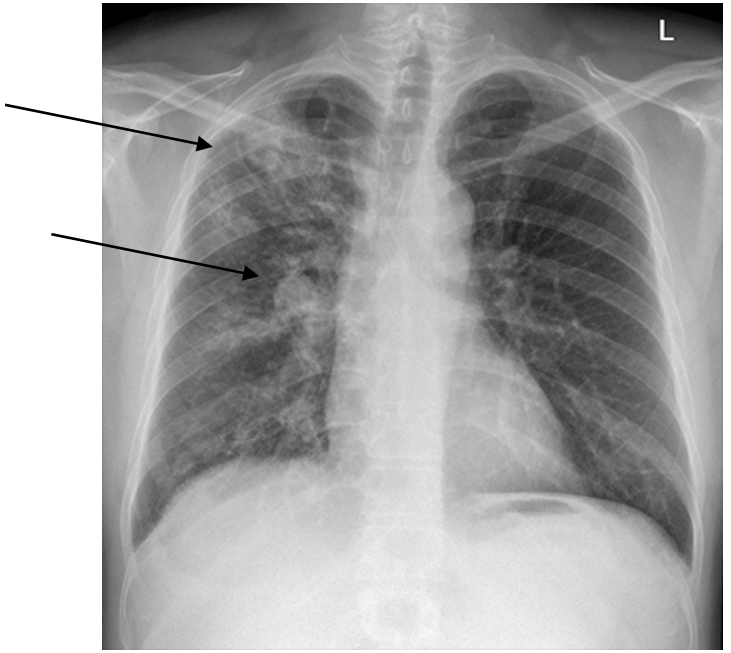


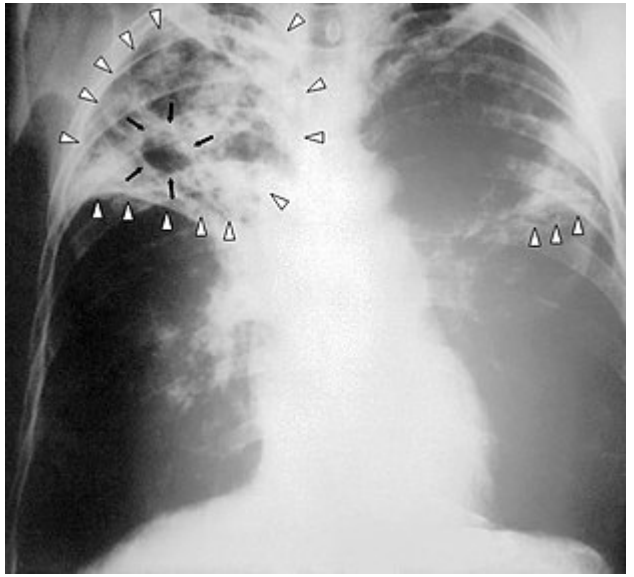
Fig. 6 Current TB diagnostic pathway (left) and potential diagnostic pathways to optimise rapid diagnosis (right) including WGS and additional molecular testing if resources allow

Park et al. BMC Infectious Diseases (2022) 22:349

Imaging

- A posterior-anterior view is the standard (apical and posterior segments of the upper lobe or in the superior segments of the lower lobe)
- A lateral view may be helpful, especially in children.
- Children: abnormalities tend to be minimal (mostly lymphadenopathy).
- Atypical lesions especially in HIV-infected and immunosuppressed (Lesions anywhere in the lungs and may differ in size, shape, density, and cavitation).
- Cavitary lesions usually in patients with higher CD4 counts (cavitation is thought to occur as a result of the immune response).





In patients with symptoms and signs of TB disease, a negative chest radiograph result

DOES NOT

exclude TB disease

Case

- 17 yo male in rural MN with chest mass evaluation and intermittent night sweats, otherwise healthy
- Immigrated to U.S. with family in 2012 from Thailand. Never left U.S. since. Lives with family, attends school.
- No known TB exposures, positive TB test or prior treatment. BCG vaccine status unknown.

  - QuantiFERON Negative
  - Lung nodules noted on chest CT, cervical and bilateral axillary lymph nodes.
  - Sputum samples were AFB smear negative, culture negative, GeneXpert MTB/RIF negative
  - Ultrasound guided FNA of chest mass: Negative for malignancy. AFB stain negative, culture pending
  - No PCR was ordered on fresh chest mass tissue.
  - LDT Mtb PCR performed on FFPE Pathology Block tissue – negative;

What would you do next?

1. Start RIPE
2. No treatment, wait for cultures
3. Do more tests

Thank you