



Laboratory Services
TB or Not TB? That is the question.

Reeti Khare, PhD, D(ABMM)
Associate Professor, Department of Medicine
Director of Mycobacteriology and Microbiology Labs
National Jewish Health
Denver TB course
April 2025



Conflicts of Interest



- None
- *Note: I will be discussing specific commercial assays where they are endorsed by regulatory agencies (e.g. FDA or WHO) or discussed in publications but have no financial agreements.*

Learning Objectives



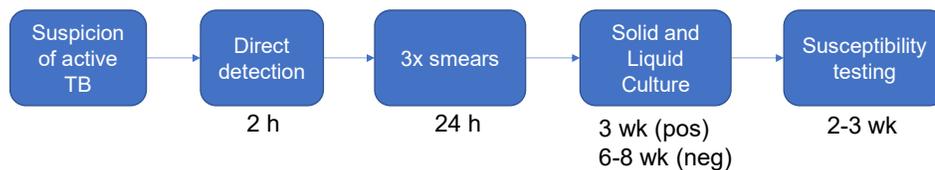
- Understand TB testing algorithm
- Assess direct testing for TB (from specimens)
- Review culture and molecular tools for identification
- Evaluate phenotypic and gene-based susceptibilities

Importance of Diagnosis

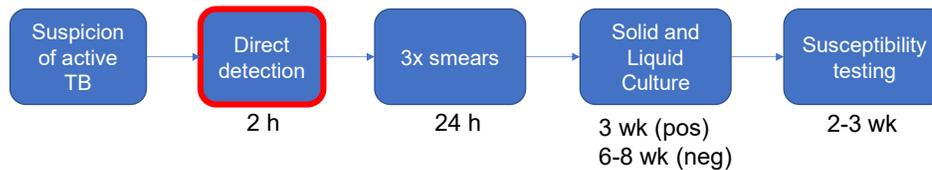


- Of the 7 million global TB cases, only ~55-60% of them are microbiologically confirmed (others are clinically confirmed)
- Lab testing can help find the missing cases
 - Reduce delay to diagnosis
 - Detection of drug resistance and better align treatment
 - Ultimately: reduce cases, reduce deaths

Typical testing algorithm



Typical testing algorithm



Lewinsohn et al. Official American Thoracic Society/Infectious Diseases, Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis. January 15, 2017;64(2):111-115



TB-NAAT assays



- NAAT: nucleic acid amplification test
- Cepheid GeneXpert MTB/Rif
 - Real-time PCR, 2 min hands on time, ~2 h TAT.
 - **MTB Detected/Not Detected**
 - **Report and rifampin resistance**
- MTB/Rif Ultra:
 - ~30 min faster
 - Includes additional real-time PCR targets for rifampin resistance coverage.
 - Semi-quantifies
 - LoD is ~7x lower; 5-10% more sensitive



https://www.cepheid.com/en_US/tests/Critical-Infectious-Diseases/Xpert-MTB-RIF

<https://p.widencdn.net/nvolny/Cepheid-Xpert-MTB-RIF-Ultra-Comparison-Flyer-CE-IVD-3094-English>



The Cepheid GeneXpert MTB/RIF test



- A) Has close to 100% sensitivity from sputum samples (overall)
- B) Has poor specificity
- C) Can only be performed on isolates
- D) Is only FDA-approved for sputum, not any other sources

The Cepheid GeneXpert MTB/RIF test



- A) Has close to 100% sensitivity from sputum samples (overall)
- B) Has poor specificity
- C) Can only be performed on isolates
- D) Is only FDA-approved for sputum, not any other sources

Performance Characteristics



Source	Population	Sensitivity (%)	Specificity (%)
Sputum/ Pulmonary	Adult	85 (81 for HIV+, 67 for smear neg)	98
	Children	65 (72 for HIV+)	99

WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection, Jun 2020



Performance Characteristics



Source	Population	Sensitivity (%)	Specificity (%)
Sputum/ Pulmonary	Adult	85 (81 for HIV+, 67 for smear neg)	98
	Children	65 (72 for HIV+)	99
Gastric aspirate	Children	73	98-99
Pleural fluid	Adults	50	99
Peritoneal fluid	Adults	59	97
Cerebrospinal fluid	Adults	70	97
Synovial fluid	Adults	97	94
Lymph node aspirate	Adults	89	86
Lymph node biopsy	Adults	82	79
Urine	Adults	85	97

WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection, Jun 2020



TB-NAAT assays



- WHO endorsed assay: Molbio Truenat: MTB and MTB Plus
 - Real-time PCR using a microchip
 - Detects *M. tuberculosis*
 - Followup assays can be performed sequentially for
 - Rif resistance.
 - Inh resistance
 - Multistep, ~1 hour per test
 - Portable, battery-powered



<https://tbfacts.org/truenat/>

Assays for Direct TB Diagnosis

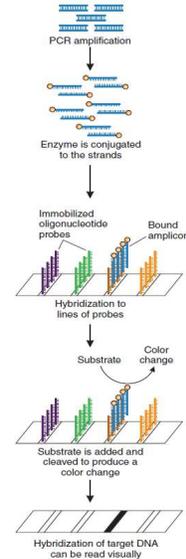


- 5 groups of technologies
 - Real-time PCR
 - Line probe assays
 - LAMP
 - Antigen detection
 - Targeted next generation sequencing

Line Probe Assays (LPAs)

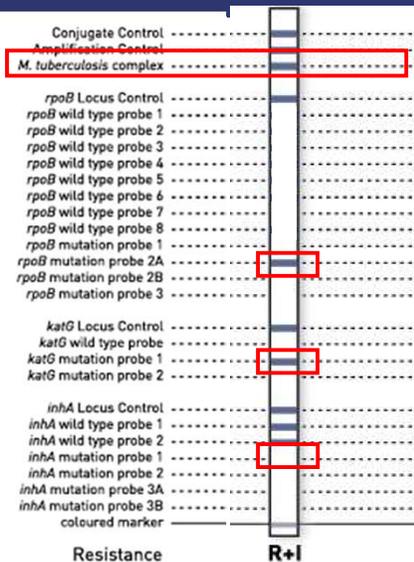


- Step 1: PCR
- Step 2: Amplicons are bound onto a membrane containing capture probes for identification and drug resistance genes
- Step 3: Pattern of binding is read



Khare. Guide to clinical and Diagnostic Virology, 2019

Line Probe Assays (LPAs)



<https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/genotype-mtbdplus.html>

Assays for Direct TB Diagnosis



- 5 groups of technologies
 - Real-time PCR
 - Line probe assays
 - LAMP
 - Antigen detection
 - Targeted next generation sequencing

LAMP



- Loop-mediated isothermal amplification (e.g. Loopamp)
- Complex
- Isothermal:
 - No thermocycler needed.
 - Less energy intensive: can be battery powered, or even in a water-bath.

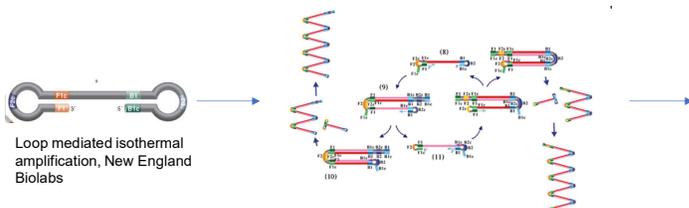
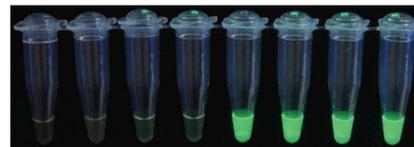


Figure 2. Visual display of TB-LAMP results under ultraviolet light



Performance Characteristics



Diagnosis of pulmonary TB in adults

Test	Version	Sensitivity (%)	Specificity (%)	Detection of rifampin resistance
Cepheid GeneXpert	MTB/RIF	85 (81 for HIV+)	97-98	Y
	MTB/RIF Ultra	90	96	Y
Molbio Truenat	MTB	73-83	98-99	N
	MTB Plus	80-89	96-98	N
	MTB-Rif	-	-	Y (93% sens; 96% spec)
TB-LAMP		74-78 (64 for HIV+)	98-99	N

- Rowlinson, Musser and Khare, Mycobacterium tuberculosis Complex, in Manual of Clinical Microbiology, 2023
- WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection, Jun 2020
- WHO Operational Handbook on Tuberculosis, Module 3. Diagnosis - Rapid diagnostics for tuberculosis detection, Jun 2020.
- Rapid Communication: Molecular assays as initial tests for the diagnosis of tuberculosis and rifampin resistance. 2020 Jan, World Health Organization.
- The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis. Policy Guidance, World Health Organization. 2016



A NAAT should be used at the end of treatment to assess whether the patient has been cured of TB

- A. True
- B. False





A NAAT should be used at the end of treatment to assess whether the patient has been cured of TB

A. True

B. False

Assays for Direct TB Diagnosis



- 5 groups of technologies
 - Real-time PCR
 - Line probe assays
 - LAMP
 - Antigen detection
 - Targeted next generation sequencing

Antigen Testing



- Mycobacterial glycolipid called lipoarabinomannan (LAM)
- Excreted in urine
- Abbott-Alere Determine TB LAM Ag – Lateral Flow Assay
 - 25 min
 - **Generally poor sensitivity and specificity (~42%)**
 - Slightly better performance in select populations (TB-HIV co-infections, ~77%)
 - TB dissemination and renal involvement of infection may be the mechanism



<https://www.globalpointofcare.abbott/ww/en/product-details/determine-tb-lam.html>

Lateral Flow Urine Lipoarabinomannan Assay for the Diagnosis of Active Tuberculosis in people living with HIV. New Diagnostic Tests Policy Update. 2019



Assays for Direct TB Diagnosis



- 5 groups of technologies
 - Real-time PCR
 - Line probe assays
 - LAMP
 - Antigen detection
 - Targeted next generation sequencing



Sequencing



- Sanger sequencing
- Next generation sequencing (NGS)
- Whole genome sequencing
- Targeted NGS

Sequencing



- Sanger sequencing: sequences a small portion of DNA. It is a technique
- Next generation sequencing (NGS): massively parallel sequencing. It is a technique.
- Whole genome sequencing: applies NGS techniques to sequence all of an organism's genome
- Targeted NGS: applies NGS techniques to sequence some of an organism's genome.

Targeted Next Generation Sequencing



- Detects MTB directly from sputum and isolates
 - Reduces turnaround time
 - Simultaneous detection of resistance factors

MTBC Culture	MTBC Identification by GeneLEAD (Diagenode, Belgium)		MTBC Identification by Deeplex (Genoscreen, France)	
	Positive	Negative	Positive	Negative
Positive	58	0	46	12
Negative	5	49	0	54
Sensitivity	100		79	
Specificity	98		100	

Bonnet et al. A Comprehensive Evaluation of GeneLEAD VIII DNA Platform Combined to Deeplex Myc-TB® Assay to Detect in 8 Days Drug Resistance to 13 Antituberculous Drugs and Transmission of *Mycobacterium tuberculosis* Complex Directly From Clinical Samples. *Front Cell Infect Microbiol.* 2021



Assays for Direct TB Diagnosis

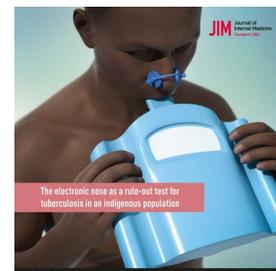


- 5 groups of technologies
 - Real-time PCR
 - Line probe assays
 - LAMP
 - Antigen detection
 - Targeted NGS

African Giant Pouched Rat



<https://www.nationalgeographic.org/article/giant-rats-trained-sniff-out-tuberculosis-africa/>



<https://onlinelibrary.wiley.com/doi/10.1111/joyim.13281>

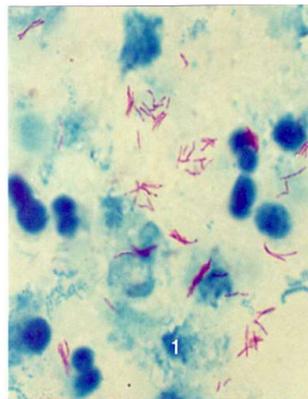
Biewer et al. Diagnostic accuracy of an exhaled breath test for TB in hospitalized patients with cough or risk, *International Union Against Tuberculosis and Lung Disease*, 2024



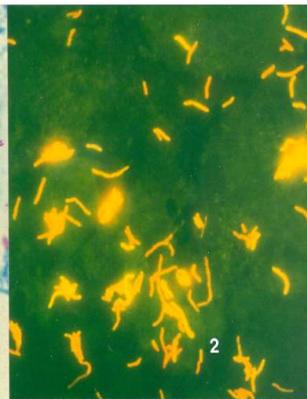
AFB Smears



AFB Smears



Light microscopy
(Ziehl-Neelsen staining)



Fluorescence microscopy
(Auramine-O staining)

Fluorescent AFB smears should be



- A) Performed due to their high sensitivity and specificity
- B) Performed to provide an estimate of bio-burden
- C) Discontinued due to their low sensitivity and specificity
- D) Discontinued because they capture NTM as well as TB

Fluorescent AFB smears should be



- A) Performed due to their high sensitivity and specificity
- B) Performed to provide an estimate of bio-burden
- C) Discontinued due to their low sensitivity and specificity
- D) Discontinued because they capture NTM as well as TB

AFB smears



- Ziehl Neelsen: sensitivity = 20-70%; need $\sim 10^4$ - 10^5 CFU/ml
- Auramine-rhodamine smears: ~ 5 -10% more sensitive

Somoskovi et al. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2925666/#R2>
Cattamanchi et al. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2754584/>
Singh, Parija. <https://www.ncbi.nlm.nih.gov/pubmed/10772577>
Azadi et al. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5897959/>
Ghiasi et al. <https://link.springer.com/article/10.1007/s40475-015-0043-1>

Benefits/Limitations of AFB smears



- Disadvantage: low sensitivity
 - “TB programmes should transition to replacing microscopy as the initial diagnostic test with molecular [WHO-endorsed rapid diagnostics] that allow for the detection of MTBC.”
- Advantages:
 - Turnaround time: 24 hours
 - Can detect NTM
 - Are semiquantitative

Culture



Culture still needs to be performed if a NAAT is positive

- A. True
- B. False



Culture still needs to be performed if a NAAT is positive

A. True

B. False

Culture



- Limitations
 - Slow TAT
 - highly trained personnel
- Advantages
 - High specificity
 - Can identify false positives and false negatives from molecular testing
 - Can identify NTM
 - Isolate needed for drug susceptibility testing

Specimen Collection



- Location of disease (Pulmonary specimens are the most common)
- Time of collection (Early morning sputum samples better than random specimens)
- Ease of collection
 - Pediatric patients: gastric lavage
 - Biohazards (e.g. risk of aerosolization with sputum collection)

Study	Random specimen positive (%)	Early morning specimen positive (%)
Abraham et al. [10] (smear positivity)	21/49 (43)	32/49 (65)
Ssengooba et al. [11] (MGIT culture positivity)	12/21 (57)	21/21 (100)

Caulfield, Wengenack. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques
Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, Volume 4, August 2016, Pages 33-43



Culture Techniques



- Specimen processing
 - Digestion: N-acetyl-L-Cysteine (NALC)
 - Decontamination: 2% NaOH
 - Concentration: centrifugation
- Liquid media
 - 10-15% more sensitive than solid cultures.
 - TAT: ~10 days for a positive
- Solid media
 - TAT: 20-25 days for a positive
 - Lowenstein Jensen agar
 - contains egg and malachite green
 - Middlebrook agar
 - Contains casein hydrolysate (for MDR TB)
- Pyruvate needed instead of glycerol for growth of *M. bovis*
- "Rough and buff" colonies
- Cultures go for 6-8 weeks



<https://www.fishersci.ca/shop/products/lowenstein-jensen-medium-lj/p-4523753>

Middlebrook 7H11



<https://www.fishersci.ca/shop/products/remel-middlebrook-7h11-agar/r01605>



Isolate Confirmation

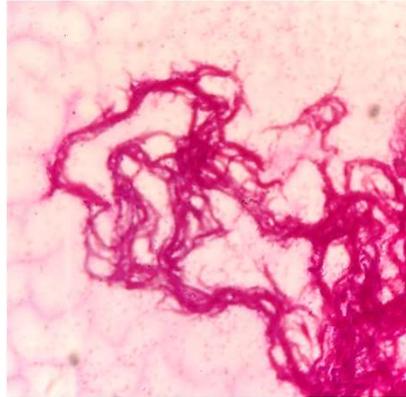


Photo: Valerie Rodriguez

Methods of identification



- MALDI-TOF mass spectrometry
 - Advantages: fast, low cost of operation, instrumentation may already be in the lab, good coverage of many mycobacteria.
 - Limitations: Does not differentiate some mycobacterial complexes; does not subspeciate; does not detect drug resistance markers



Line Probe Assays



Specimen	Assay name	Identification of MTB	Resistance markers	Company
Sputum only	GenoType CMdirect Ver 1.0	Yes (also ID's ~20 NTM)	No	Hain
	Genoscholar NTM+MDRTB II (Previously called NTM+MDRTB Detection Kit 2)	Yes (also identifies 3 other NTM)	Yes (rpoB, inhA, katG)	Nipro
Sputum or culture	GenoType MTBDRplus 2.0	Yes	Yes (rpoB, inhA, katG)	Hain
	Genotype MTBDRsl ver 1.0 (e)	Yes	Yes (rrs, gyrA, embB)	Hain
	Genotype MTBDRsl ver 2.0 (e)	Yes	Yes (rrs, gyrA, gyrB, eis)	Hain
	INNO-LiPA Mycobacteria v2	Yes (also ID's 16 NTM)	Yes (Rif)	Fujirebio
Culture only	GenoType Mycobacterium CM Ver 2.0	Yes (also ID's ~20 NTM)	No	Hain
	GenoType MTBC Ver 1.X	Yes (also differentiates the MTBC)	No	Hain



TB Complex Species



Complex members	Generally Adapted to (not exclusive)
<i>M. tuberculosis</i>	Humans
<i>M. bovis</i>	Domestic and wild bovine animals, humans
<i>M. bovis</i> BCG	Culture, immunocompromised humans
<i>M. africanum</i>	Humans – limited to Africa
<i>M. canetti</i>	Humans – rare
<i>M. caprae</i>	Goats
<i>M. orygis</i>	Various
<i>M. microti</i>	Small rodents
<i>M. pinnipedii</i>	Seals/walruses
<i>M. suricattae</i>	African mammals (Meerkats)
<i>M. mungi</i>	African mammals (Banded mongooses)
Dassie bacillus	African mammals (Rock hyraxes)

TB Complex Species



Complex members	Generally Adapted to (not exclusive)
<i>M. tuberculosis</i> ~95%	Humans
<i>M. bovis</i> ~2%	Domestic and wild bovine animals, humans
<i>M. bovis</i> BCG ~1%	Culture, immunocompromised humans
<i>M. africanum</i> ~2%	Humans – limited to Africa
<i>M. canetti</i>	Humans – rare
<i>M. caprae</i>	Goats
<i>M. orygis</i>	Various
<i>M. microti</i>	Small rodents
<i>M. pinnipedii</i>	Seals/walruses
<i>M. suricattae</i>	African mammals (Meerkats)
<i>M. mungi</i>	African mammals (Banded mongooses)
Dassie bacillus	African mammals (Rock hyraxes)

Pyr R, tracing (animals or iatrogenic)

Clarke et al. Animal-adapted members of the *Mycobacterium tuberculosis* complex endemic to the southern African subregion, J S Afr Vet Assoc. 201



Other Confirmatory Tests



- Microscopy
- MALDI-TOF MS
- NAATs (e.g. Line probe assays)
- Sequencing
 - Sanger
 - Targeted NGS
 - Whole genome sequencing



Antimicrobial Susceptibility Testing



Lewinsohn et al. Official American Thoracic Society/Infectious Diseases, Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis. January 15, 2017;64(2):111-115



Updated definitions



Term	Abbreviation	Resistance
Rifampin resistant TB	RR-TB	Rif
Multidrug resistant TB	MDR-TB	Rif and Inh
Pre-extensively drug-resistant TB	Pre-XDR TB	Rif (with or without Inh) and a FQ
Extensively drug-resistant TB	XDR TB	Pre-XDR And at least one "Group A drug" (levofloxacin, moxifloxacin, bedaquiline, linezolid)

<https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis>



Antimicrobial Susceptibility Testing



- Gene-based testing from specimen or isolate
- Phenotypic testing from an isolate

Global tuberculosis report 2019. WHO

Gene-based AST



- Pros:
 - Faster! (hours-days instead of 6-8 weeks of culture based AST)
 - Some well characterized mutations that correlate well with phenotypic AST
 - Recommended by the WHO in some cases

Relevant genotypic ASTs for TB



Use	Drug	Genes containing mutations associated with drug resistance
1st line	Rifampin	<i>rpoB</i>
	Isoniazid	<i>inhA, katG, ahpC</i>
	pyrazinamide	<i>pncA</i>
	Ethambutol	<i>embB</i>
2nd line	Streptomycin	<i>rrs, gidB, rpsL</i>
	Amikacin	<i>rrs</i>
	Capreomycin	<i>rrs, tlyA</i>
	Kanamycin	<i>rrs, eis</i>
	Fluoroquinolones	<i>gyrA, gyrB</i>
	Linezolid	<i>rrl, rplC</i>
	Bedaquiline	<i>Rv0678, atpE, pepQ</i>
	Clofazimine	<i>Rv0678</i>
Pretomanid	<i>fgd1, fbiA, fbiB, and fbiC</i>	

- CDC, 2022.
<https://www.cdc.gov/tb/topic/drtb/bpal/default.htm#Microbiologic-monitoring>
 - Deeplex User Manual, Genoscreen, V3, July 2020

Genotypic detection of rifampin resistance:



- A. Is not available in most labs
- B. May represent a false positive
- C. Occurs using the *rif* gene
- D. Should be ignored



Genotypic detection of rifampin resistance:

- A. Is not available in most labs
- B. May represent a false positive
- C. Occurs using the *rif* gene
- D. Should be ignored

Gene-based AST



- Real-time PCR
 - Cepheid: MTB/Rif and MTB/Rif Ultra
 - Truenat: First MTB, then Rifampin
 - Cepheid: MTB/XDR: *Inh* and second line drugs

Drug Resistance	Gene Target
isoniazid	<i>inhA</i> promoter
	<i>katG</i>
	<i>fabG1</i>
	<i>oxyR- ahpC</i>
ethionamide	intergenic region
	<i>inhA</i> promoter
fluoroquinolones	<i>gyrA</i>
	<i>gyrB</i>
amikacin, kanamycin, capreomycin	<i>rrs</i>
	<i>eis</i> promoter

Gene-based AST



- Line probe assays

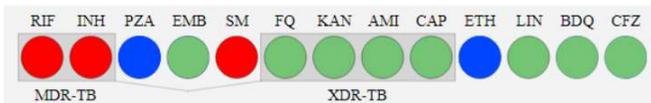
Specimen	Assay name	Drugs	Resistance markers	Company
Sputum or culture	Genoscholar NTM+MDRTB II (Previously called NTM+MDRTB Detection Kit 2)	Rifampin AND isoniazid	Yes (rpoB, inhA, katG)	Nipro
	GenoType MTBDRplus 2.0	Rifampin AND isoniazid	Yes (rpoB, inhA, katG)	Hain
	Genotype MTBDRsl ver 1.0 (e)	Aminoglycosides, fluoroquinolones, ethambutol	Yes (rrs, gyrA, embB)	Hain
	Genotype MTBDRsl ver 2.0 (e)	Fluoroquinolones, second line injectables	Yes (rrs, gyrA, gyrB, eis)	Hain
Culture only	INNO-LiPA Mycobacteria v2	Rifampin	Yes (Rif)	Fujirebio



Gene-based AST



- Whole genome sequencing
 - Lab developed tests (NY)
- Targeted NGS of resistance genes
 - Lab developed tests (CA, FL, IN, MO)
 - AmPORE TB (Oxford Nanopore, UK), TBSeq (ShengTing, CH) and Deeplex Myc-TB (GenoScreen, FR)



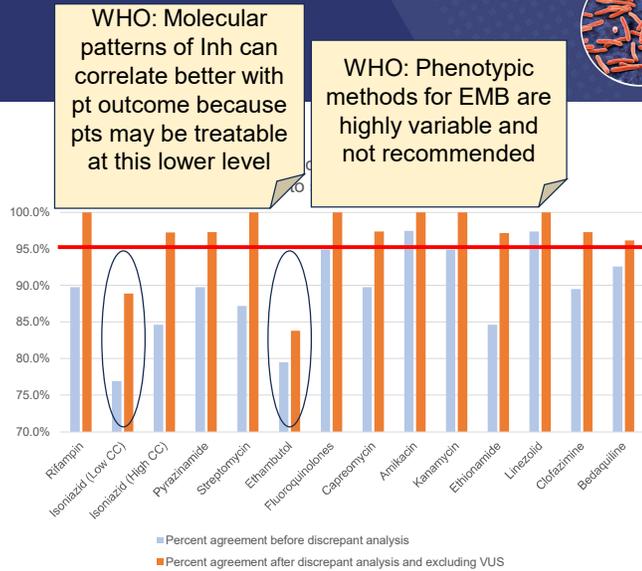
https://www.genoscreen.fr/images/genoscreen-services/deeplex/technical_note_20200706_CE.pdf

- Tuberculosis Laboratory Aggregate Report, 5th ed. 2019. https://www.cdc.gov/tb/publications/reports/articles/2019-Aggregate_Report.pdf
- Lee and Pai. Real-Time Sequencing of *Mycobacterium tuberculosis*: Are We There Yet? JCM, 2017
- Papaventsis et al. Whole genome sequencing of *Mycobacterium tuberculosis* for detection of drug resistance: a systematic review. Clin Micro and Infection, 2017



Gene-based AST

- Performance:
 - $\geq 95\%$ correlation with phenotypic assays (orange bars)



Jouet et al. Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs, Eur Resp J, 2021
 Campbell et al. Molecular detection of mutations associated with first-and second-line drug resistance compared with conventional drug susceptibility testing of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother, 2011



Antimicrobial Susceptibility Testing

- Gene-based testing from specimen or an isolate
- Phenotypic testing from an isolate
 - Advantages:
 - resistance detected even if outside common targeted genes
 - New mutations or genomic regions associated with resistance don't need to be known to detect resistance



Proportion Method



- No single isolate, No MIC
- Looks at populations of isolates in a patient
- Clinical response unlikely if >1% resistance seen at a defined “critical” concentration

Antimicrobial agent ^f	Medium and concentration(s) (µg/ml) ^a			
	Liquid systems		Agar proportion	
	MGIT 960	VersaTrek	7H10	7H11
First-line agents				
RIP ^b	1	1	1	1
INH ^b	0.1	0.1	0.2	0.2
PZA	100	300	NR ^d	NR ^d
EMB	5	5	5	7.5
Second-line agents				
INH-high ^c	0.4	0.4	1	1
Amikacin	1		4	
Capreomycin	2.5		10	10
Ethionamide	5		5	10
Kanamycin	2.5		5	6
Levofloxacin	1.5		1	
Moxifloxacin	0.25		0.5	0.5
PAS	4		2	8
Rifabutin	0.5		0.5	0.5
Streptomycin ^e	1, 4		2	2

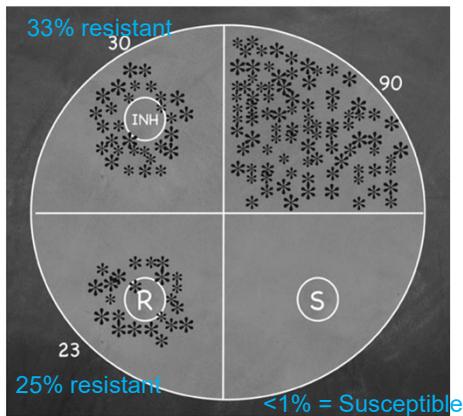
Woods et al. Susceptibility Test Methods: Mycobacteria, *Nocardia* and Other Actinomycetes. Chapter 78, Manual of Clinical Microbiology 12th edition.



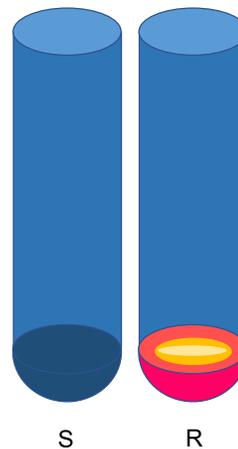
Proportion Method



Agar proportion



MGIT broth proportion



Barry and Lin. Drug Resistant TB. A survival Guide for Clinicians, 3rd ed. https://www.currytbcenter.ucsf.edu/sites/default/files/tb_sg3_cha_p3_laboratory.pdf



Change to Rifampin Critical Concentrations



Antimicrobial agent ^f	Medium and concentration(s) (µg/ml) ^a			
	Liquid systems		Agar proportion	
	MGIT 960	VersaTrek	7H10	7H11
First-line agents				
RIF ^b	0.5	1	1	1
INH ^c	0.1	0.1	0.2	0.2
PZA	100	300	NR ^d	NR ^d
EMB	5	5	5	7.5
Second-line agents				
INH-high ^e	0.4	0.4	1	1
Amikacin	1		4	
Capreomycin	2.5		10	10
Ethionamide	5		5	10
Kanamycin	2.5		5	6
Levofloxacin	1.5		1	
Moxifloxacin	0.25		0.5	0.5
PAS	4		2	8
Rifabutin	0.5		0.5	0.5
Streptomycin ^f	1, 4		2	2

New

M24S
Performance Standards for Susceptibility
Testing of Mycobacteria, *Nocardia* spp., and
Other Aerobic Actinomyces

WHO, Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine), 2021



Which is right – genotypic or phenotypic methods?



- Phenotypic assays are the gold standard,
 - may not be set at the right breakpoints
- Genotypic assays may have both
 - False positives: e.g. low prevalence or detection of silent mutations
 - False negative: mutations causing resistance that are outside of the regions detected by the assay (e.g. false negative bedaquiline on Deeplex because of lack of *atpE* coverage), interpretation based on differences in strains, novel mutations
- The answers may be different for different drugs.



Summary



- Many direct TB identification tools; becoming preferred as the initial test for TB
- Culture and molecular tools for identification becoming faster
- Phenotypic and gene-based susceptibilities more available

**Thank you for joining today's
session!**