61ST ANNUAL

# Denver TB Course

(Hybrid Event)

APRIL 2-4, 2025

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# 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

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# 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.*

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# 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

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# 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

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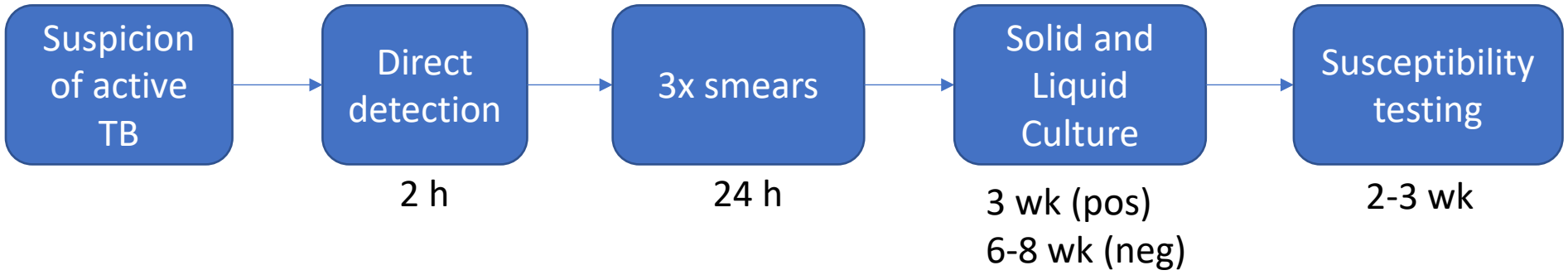
# Typical testing algorithm




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

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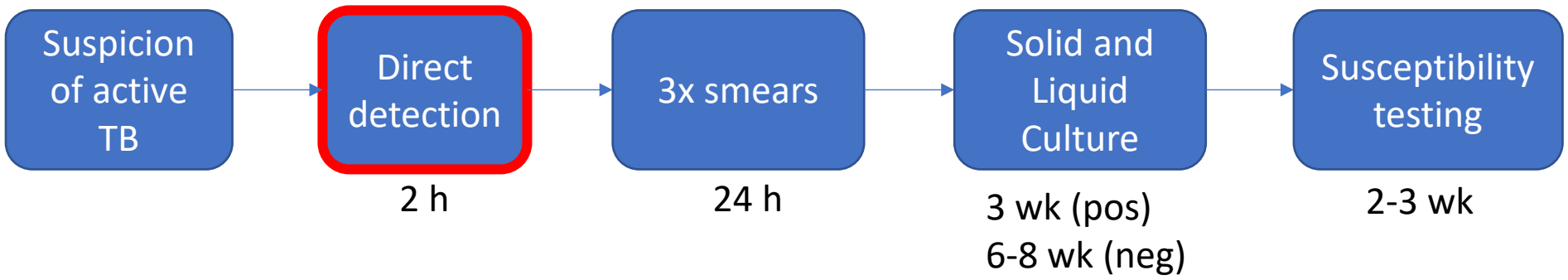
# Typical testing algorithm




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

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# 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

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# 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

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# 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

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# 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

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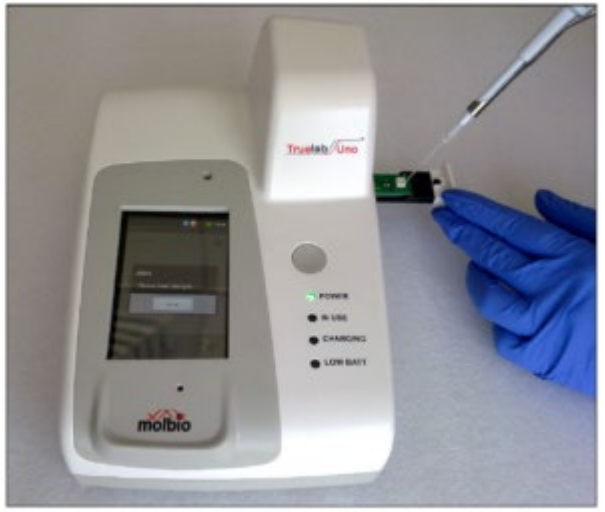
# TB-NAAT assays

- 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/

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# Assays for Direct TB Diagnosis

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

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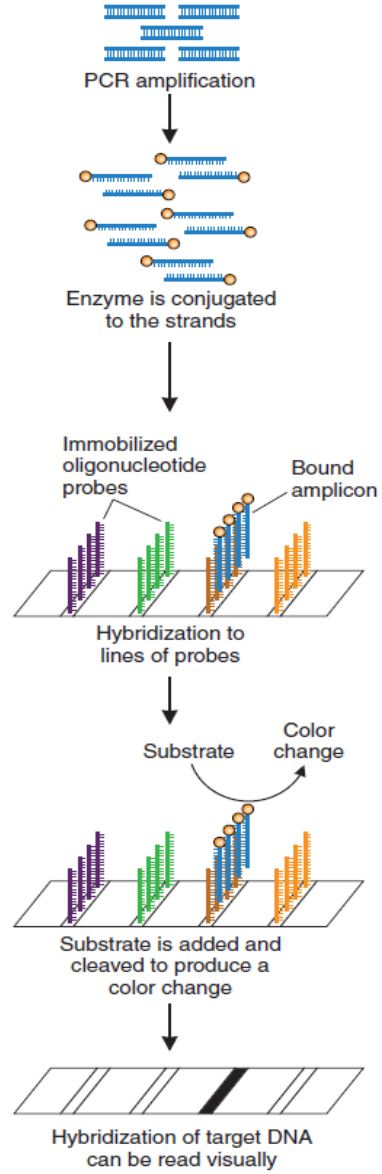
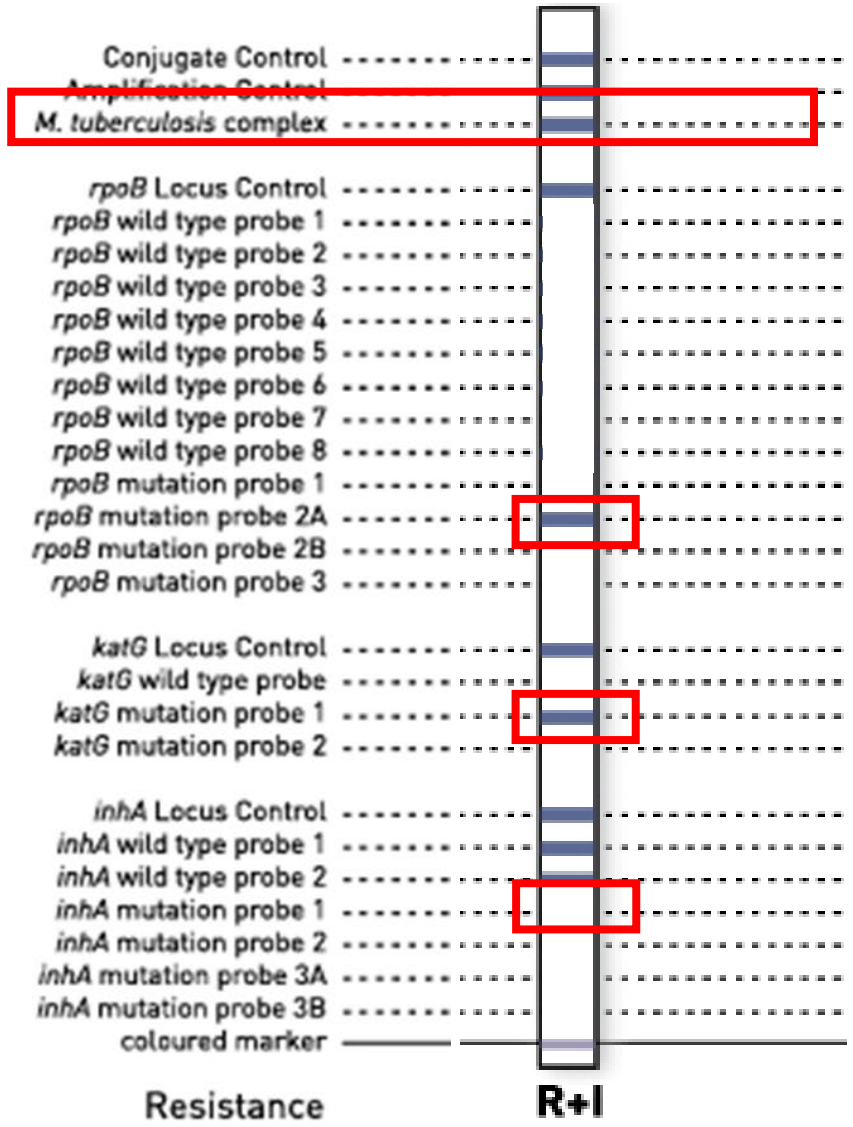
# Line Probe Assays (LPAs)

- 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-mtbdrplus.html

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# Assays for Direct TB Diagnosis

- 4 groups of technologies
  - Real-time PCR
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  - LAMP
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  - Targeted next generation sequencing

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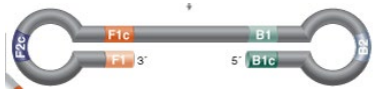
# LAMP

- 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.




Loop mediated isothermal amplification, New England Biolabs

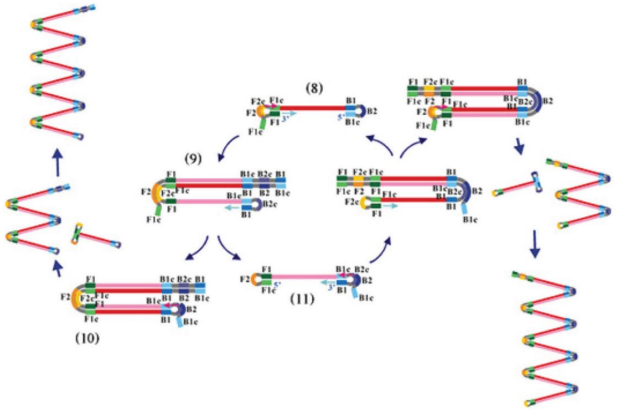
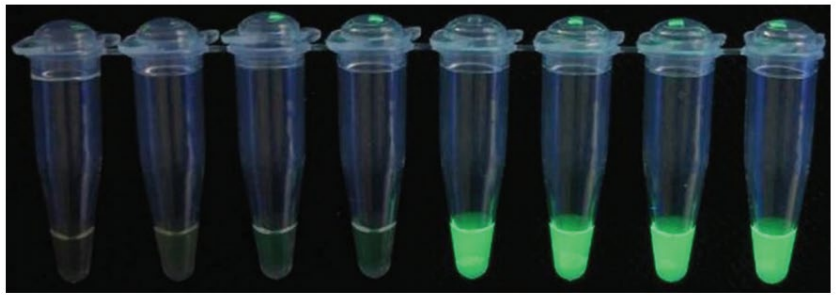



*Figure 2. Visual display of TB-LAMP results under ultraviolet light*



The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis. Policy Guidance, WHO. 2016.

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# Performance Characteristics

Diagnosis of pulmonary TB in adults

| Test | Version | Sensitivity (%) | Specificity (%) | Detection of rifampin resistance |
|---|---|---|---|---|
| Cepheid GeneXpert | MTB/RIF | 85<br>(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<br>(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 rifampicin 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.


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

A. True
B. False

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# 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

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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

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# Targeted Next Generation Sequencing

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

# Targeted Next Generation Sequencing

- 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.

| 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

- 4 groups of technologies
  - Real-time PCR
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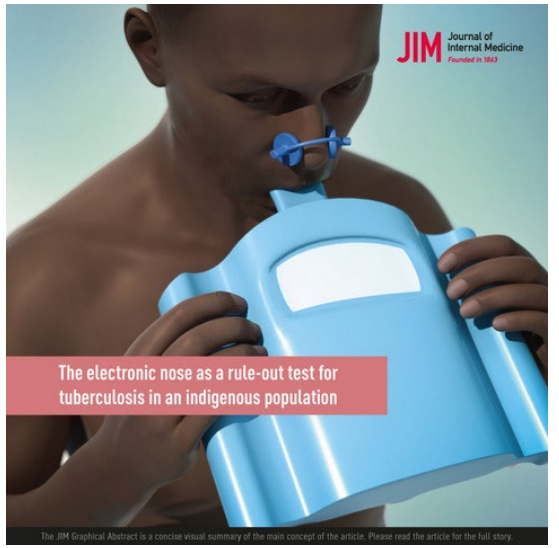
  - Targeted NGS

African Giant Pouched Rat


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




https://onlinelibrary.wiley.com/doi/10.1111/joim.13281

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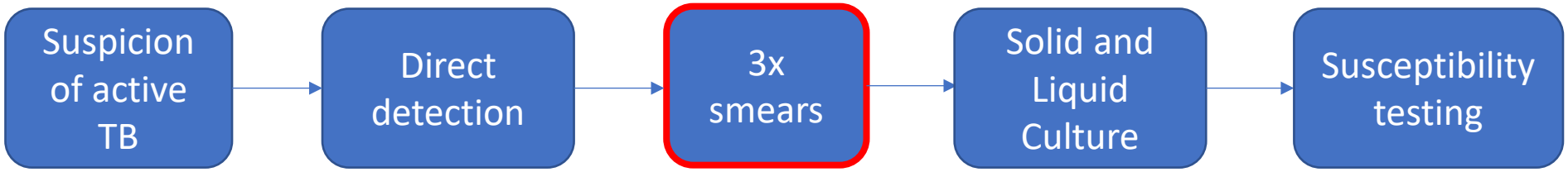
Biewer et al. Diagnostic accuracy of an exhaled breath test for TB in hospitalized patients with cough or risk, International Union Against Tuberculosis and Lung Disease, 2024

# AFB Smears

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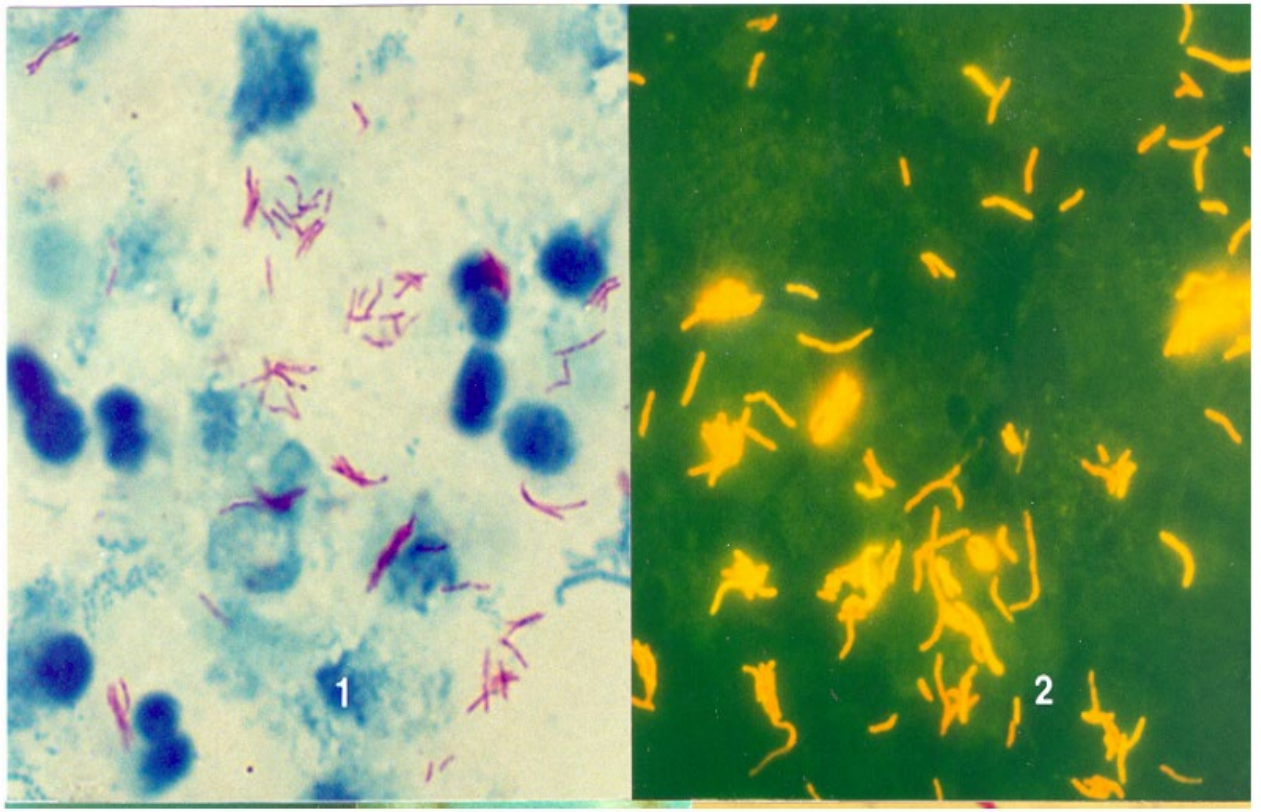
# AFB Smears




Light microscopy
(Ziehl-Neelsen staining)

Fluorescence microscopy
(Auramine-O staining)

Weyer. Prevalence Survey Preparatory Workshop, WHO Stop TB Department. Part III: Bacteriological Examination.
https://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/prevalence_survey/psws_laboratory_examination_weir.pdf

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# 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

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# AFB smears

- Ziehl Neelsen: sensitivity = 20-70%; need ~$10^4$-$10^5$ 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


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# 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

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# Culture


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# Culture still needs to be performed if a NAAT is positive

A. True
B. False

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# Culture still needs to be performed if a NAAT is positive

A. True

B. False

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# 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

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# 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)
  - Swabs are not acceptable

| 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

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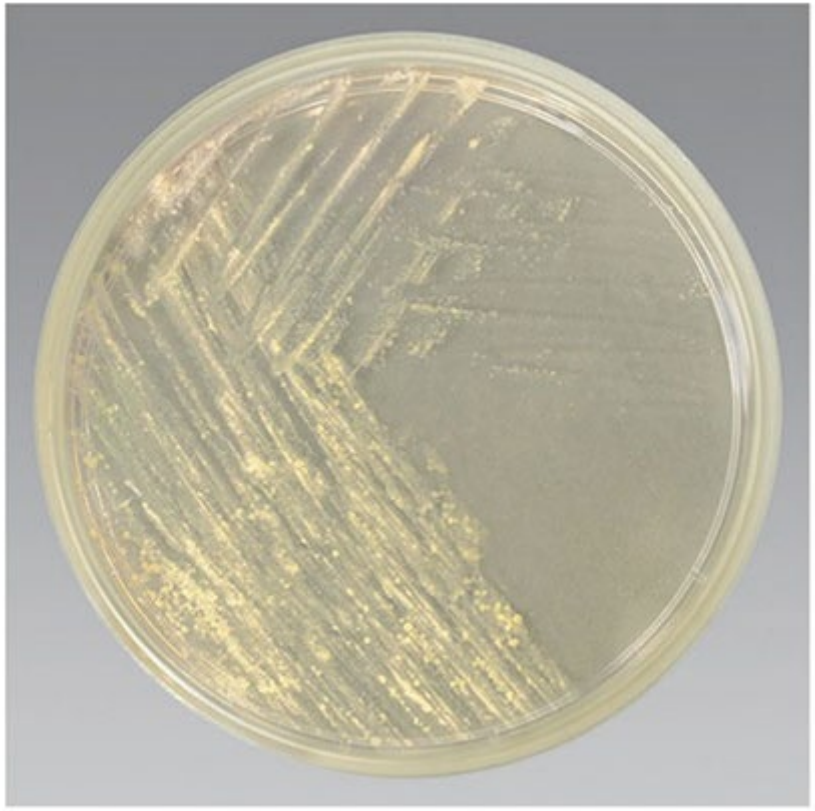
# Culture Techniques

- 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

LJ

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

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

# Isolate Confirmation

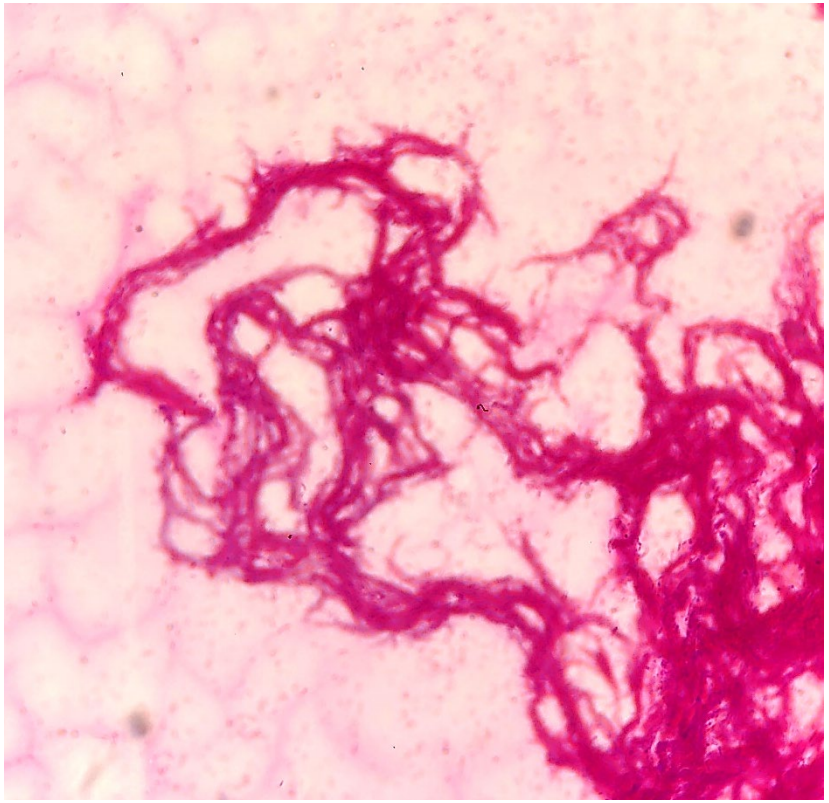


Photo: Valerie Rodriguez

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# 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

Vitek MS Prime

Vitek

Bruker

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# 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 |
| Culture only | INNO-LiPA Mycobacteria v2 | Yes (also ID's 16 NTM) | Yes (Rif) | Fujirebio |
| | 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) |
|---|---|
| ***M. tuberculosis*** | Humans |
| ***M. bovis*** | Domestic and wild bovine animals, humans |
| ***M. bovis* BCG** | Culture, immunocompromised humans |
| *M. africanum* | Humans – limited to Africa |
| *M. canetti* | Humans – rare |
| *M. caprae* | Goats |
| *M. orygis* | Various |
| *M. microti* | Small rodents |
| *M. pinnipedii* | Seals/walruses |
| *M. suricattae* | African mammals (Meerkats) |
| *M. mungi* | African mammals (Banded mongooses) |
| Dassie bacillus | African mammals (Rock hyraxes) |

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

# TB Complex Species

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

Pyr R, tracing (animals or iatrogenic)

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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
    - 16S, 23S ribosomal genes
    - *hsp65* heat shock protein
    - *rpoB* RNA polymerase
  - Targeted NGS
  - Whole genome sequencing

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# 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

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# 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<br>And at least one “Group A drug” (levofloxacin, moxifloxacin, bedaquiline, linezolid) |

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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

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# 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

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# Relevant genotypic ASTs for TB

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


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


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# 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

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# 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 | *inhA* promoter |
| | *katG* |
| | *fabG1* |
| | *oxyR- ahpC* intergenic region |
| ethionamide | *inhA* promoter |
| fluoroquinolones | *gyrA* |
| | *gyrB* |
| amikacin, kanamycin, capreomycin | *rrs* |
| | *eis* promoter |

https://cepheid.widen.net/s/cwc24p8lcl

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# 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 |

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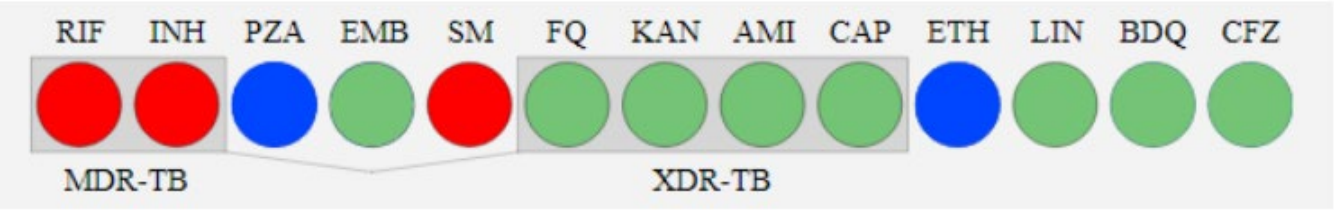
# Gene-based AST

- Whole genome sequencing
  - Lab developed tests (NY)
- Targeted sequencing 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


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

- Cons
  - Requires specialty assays (None are readily available in the US except Xpert MTB/RIF; some labs develop LDTs)
  - 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

- Sensitivity compared to phenotypic results ranges from 61% to 97%

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

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# 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
    - More resolution for higher level and lower level resistance

Global tuberculosis report 2019. WHO

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# 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

| | Medium and concentration(s) (μg/ml)[a] | | | |
|---|---|---|---|---|
| | Liquid systems | | Agar proportion | |
| Antimicrobial agent[f] | MGIT 960 | VersaTrek | 7H10 | 7H11 |
| First-line agents | | | | |
| RIF[b] | 1 | 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[e] | 1, 4 | | 2 | 2 |

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

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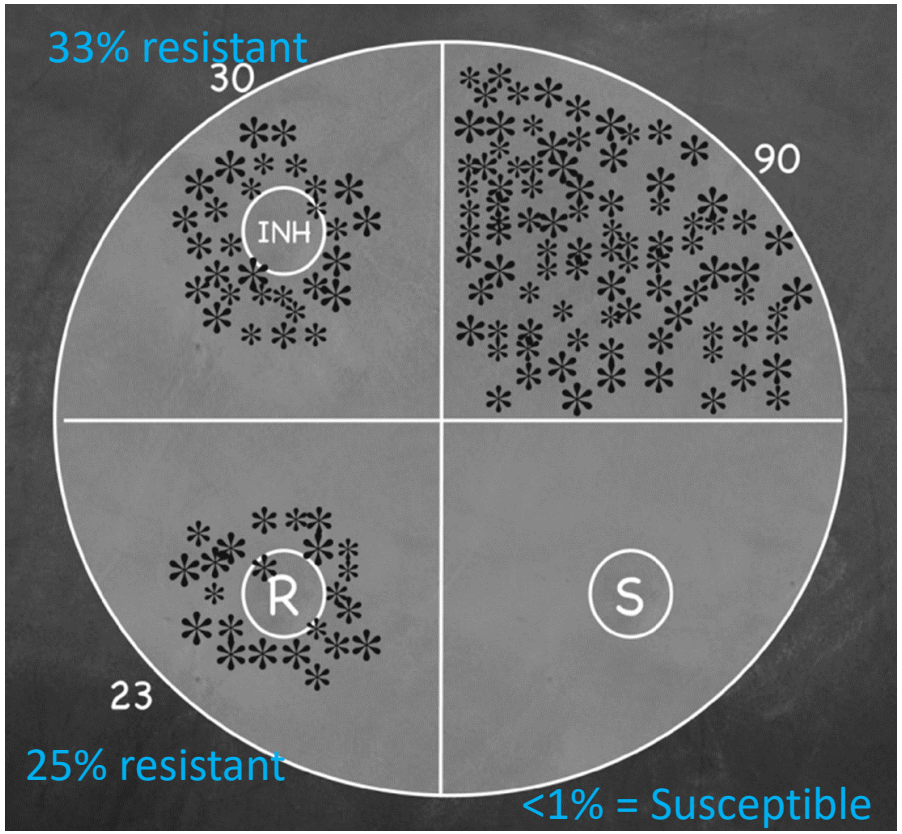
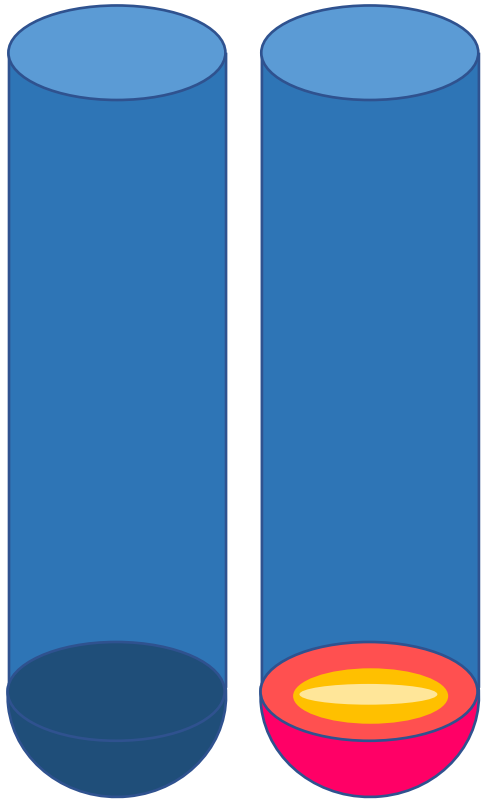
# Proportion Method

## Agar proportion




Barry and Lin. Drug Resistant TB. A survival Guide for Clinicians, 3rd ed. https://www.currytbcenter.ucsf.edu/sites/default/files/tb_sg3_chap3_laboratory.pdf

## MGIT broth proportion



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# 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~~ | 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[e] | 1, 4 | | 2 | 2 |

*New*

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

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

# What about the new drugs?

- Bedaquiline, pretomanid, linezolid, moxifloxacin
  - Bedaquiline resistance emerges quickly
- Diagnostic testing lags behind clinical use; difficult to find, limited to CDC, public health labs, or even sometimes, in the case of pretomanid, its still research use only.
- Curry Center has collated a document of places that can do testing:

https://www.currytbcenter.ucsf.edu/sites/default/files/2022-12/Full_Reference_Lab_table_12-20-22.xlsx.

https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023/featured-topics/new-treatment-tb

Saluzzo and Cirillo. Mind the gap. Rolling out new drug resistant tuberculosis regimens with limited diagnostic tools. J Clin Tuberc Other Mycobact Dis. 2023 Feb

# 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

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# Thank you for joining today's session!

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