MAYO CLINIC

MAYO CLINIC CENTER FOR TUBERCULOSIS

Diagnosis of Active Tuberculosis

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Conflicts/Disclosure

- None.

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Objectives

- Describe general approach to diagnosing active tuberculosis
- Describe clinical evaluation for diagnosing active tuberculosis
- Describe the microbiological diagnosis of active tuberculosis

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Let’s get this out of the way …

- Positive TST or IGRA does not indicate active tuberculosis
- A negative TST or IGRA does not rule out active tuberculosis

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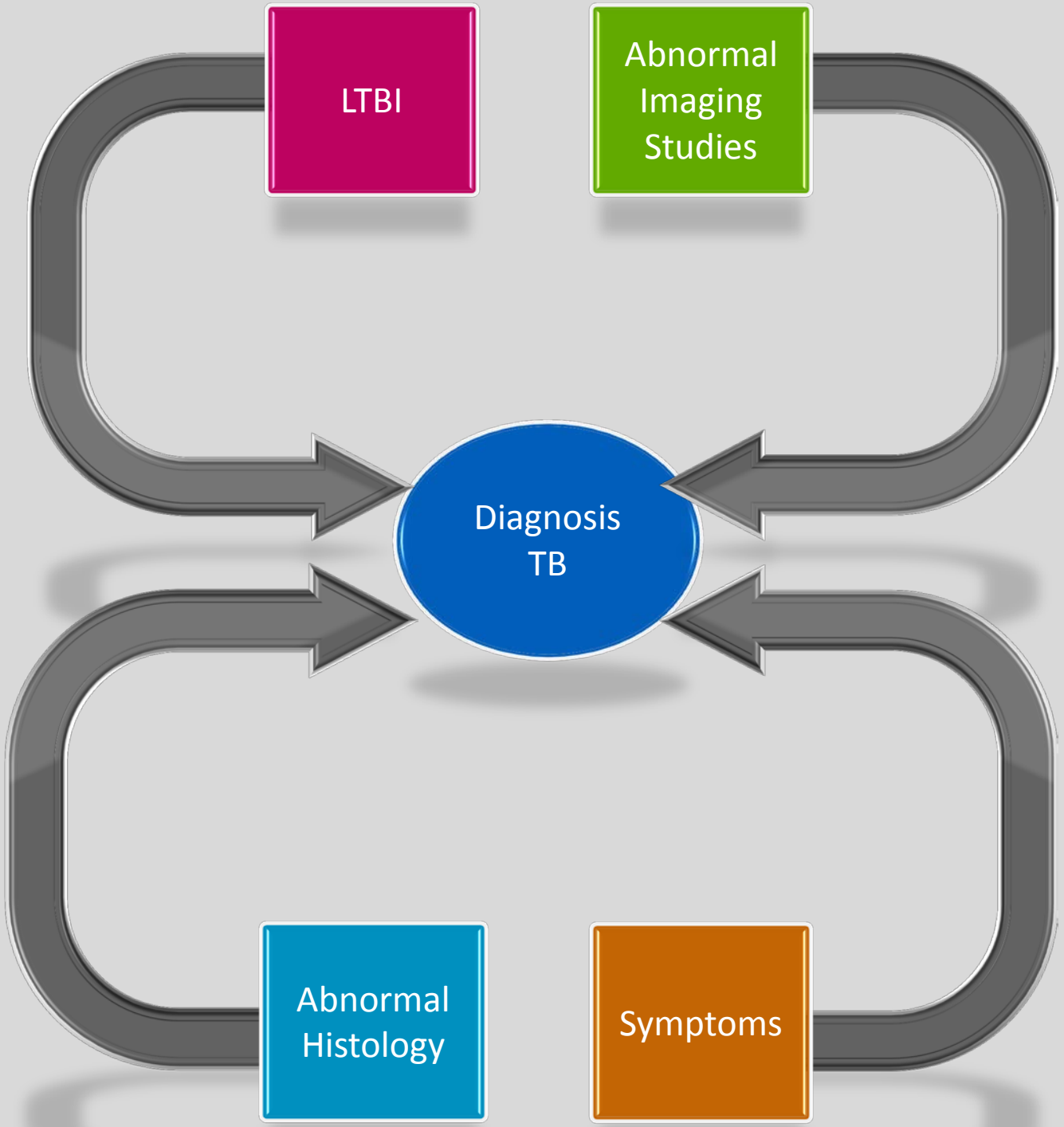
LTBI
Abnormal Imaging Studies
Diagnosis TB
Abnormal Histology
Symptoms

Evaluation for Active Tuberculosis

Medical History

Physical Exam

Radiography

Microbiologic Testing

Medical Evaluation for TB
1. Medical History

- Symptoms of disease; how long
- History of TB exposure, infection, or disease
- Past TB treatment
- Demographic risk factors for TB
- Medical conditions that increase risk for TB disease

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Persons at Risk for Developing TB Disease

Persons at high risk for developing TB disease fall into 2 categories:

- Those who have an increased likelihood of exposure to persons with TB disease
- Those with clinical conditions that increase their risk of progressing from LTBI to TB disease

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Increased Likelihood of Exposure to Persons with TB Disease

Persons at risk for exposure to persons with TB disease include:

- Close contacts to person with infectious TB
- Residents and employees of high-risk congregate settings (e.g., correctional facilities, homeless shelters, health care facilities)
- Recent immigrants from TB-endemic regions of the world (within 5 years of arrival to the United States)

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Increased Risk for Progression to TB Disease - 1

Persons more likely to progress from LTBI to TB disease include:

- HIV-infected persons
- Those with a history of prior, untreated TB or fibrotic lesions on chest radiograph
- Children $\leq$ 5 years with a positive TST

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Increased Risk for Progression to TB Disease - 2

Persons more likely to progress from LTBI to TB disease include:

- Underweight or malnourished persons
- Substance abusers (such as smoking, alcohol abusers, or injection drug use)
- Those receiving TNF-α antagonists for treatment of rheumatoid arthritis or Crohn's disease

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Increased Risk for Progression to TB Disease - 3

Persons more likely to progress from LTBI to TB disease include:

- Those with certain medical conditions such as:
  - Silicosis
  - Diabetes mellitus
  - Chronic renal failure or on hemodialysis
  - Solid organ transplantation (e.g., heart, kidney)
  - Carcinoma of head or neck
  - Gastrectomy or jejunoilial bypass

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Symptoms of Tuberculosis

Non-specific constitutional symptoms

- Loss of appetite
- unexplained weight loss
- Night sweats,
- fever
- Fatigue

Respiratory symptoms

- Prolonged cough (3 weeks or longer)
- Shortness of breath
- Hemoptysis
- Chest pain

Symptoms of possible extra-pulmonary TB

- Blood in the urine (TB of the kidney)
- Headache/confusion (TB meningitis)
- Back pain (TB of the spine)
- Hoarseness (TB of the larynx)

Evaluation for Active Tuberculosis

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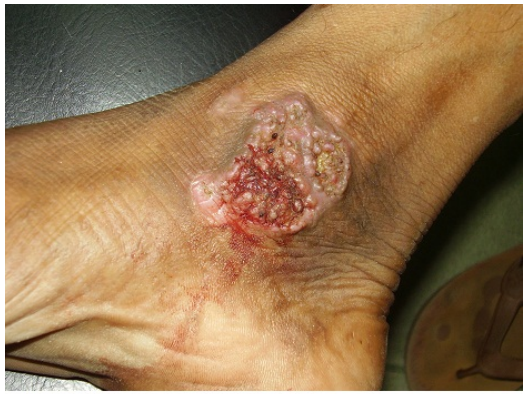
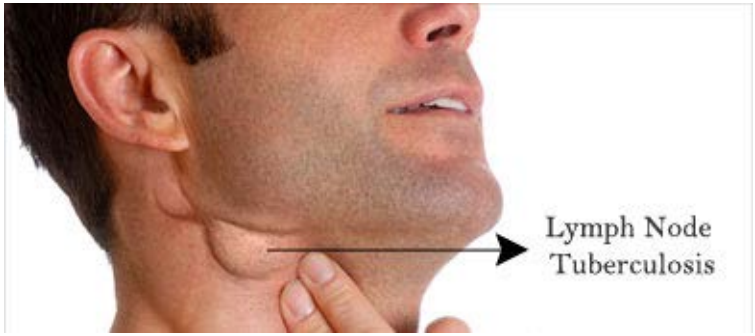
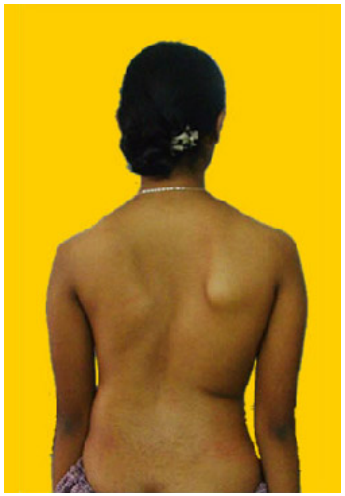
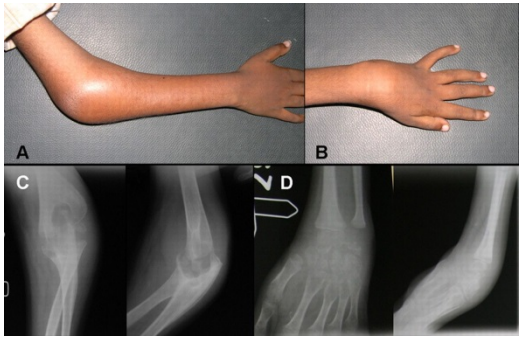
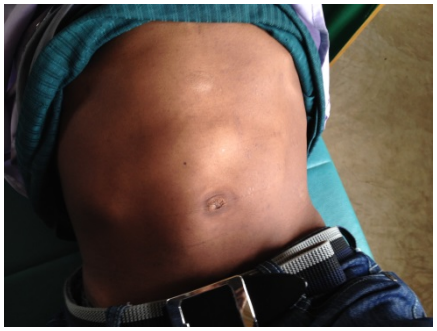


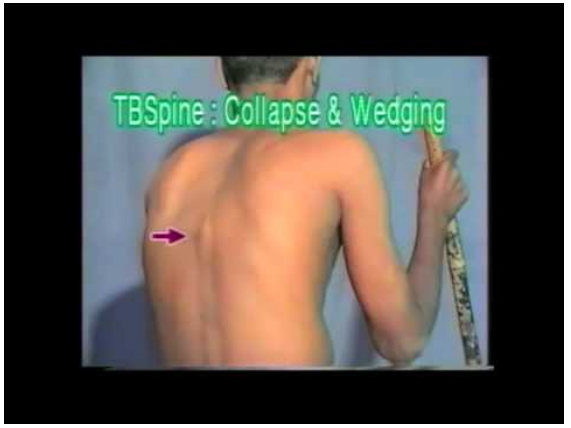
TUBERCULOSIS OF SKIN

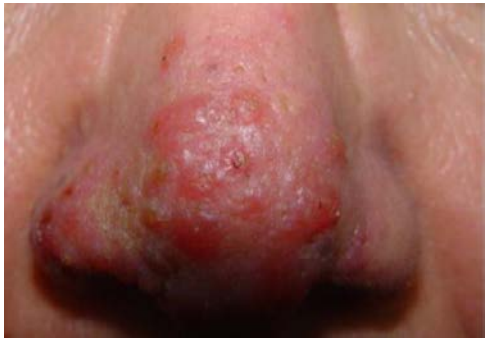


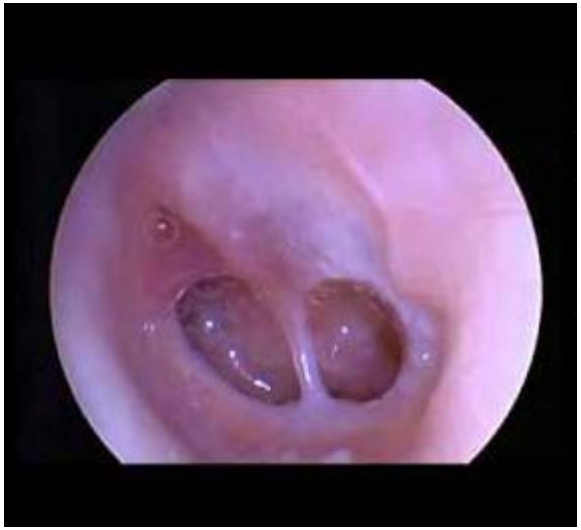


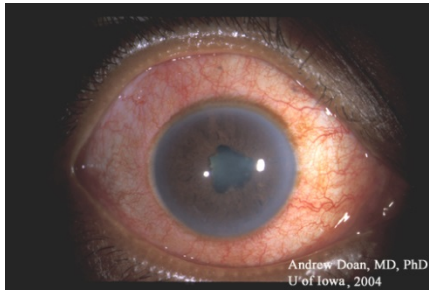












Evaluation for Active Tuberculosis

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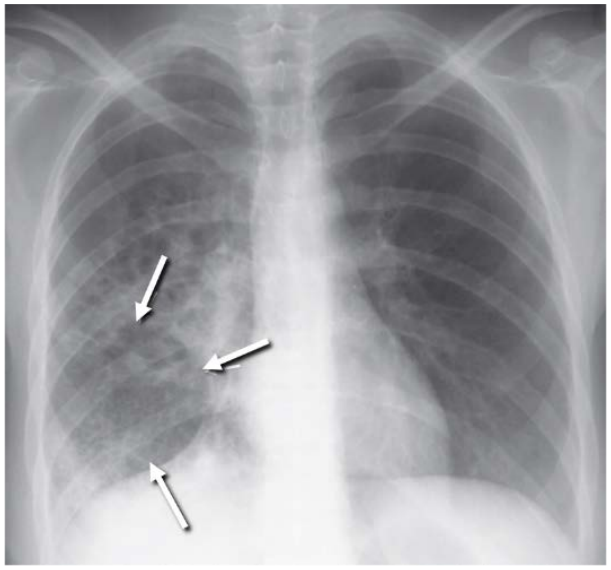

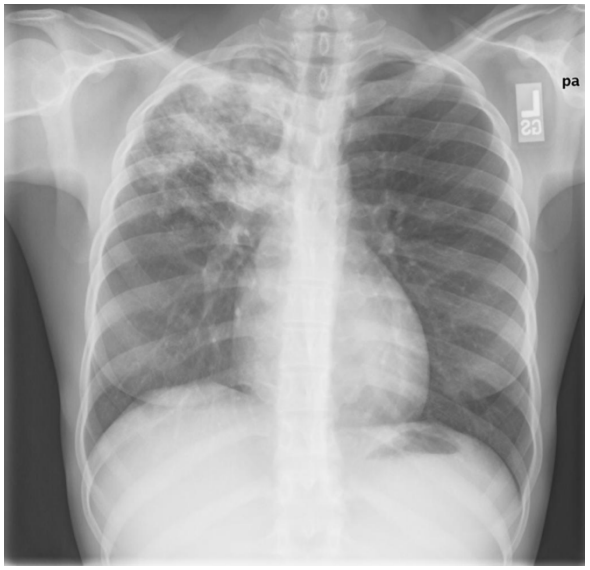
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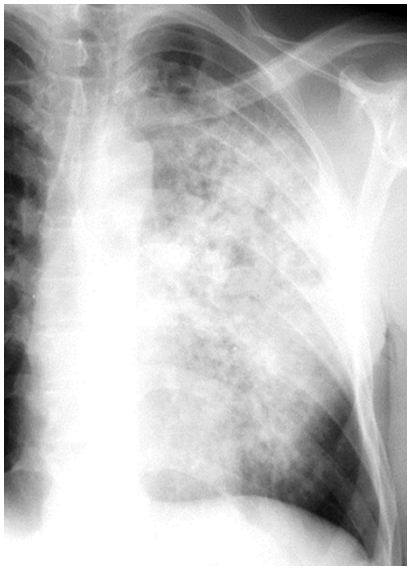

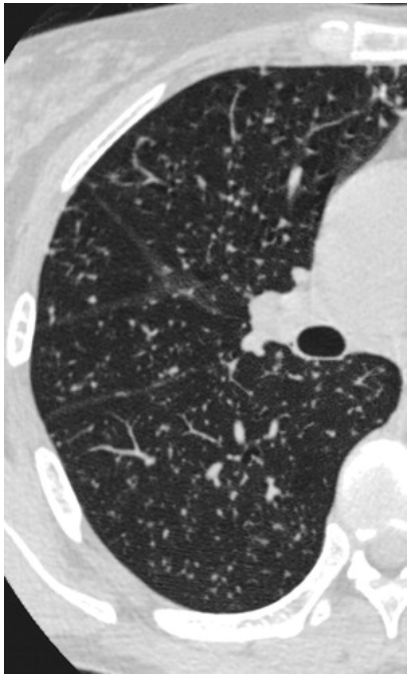

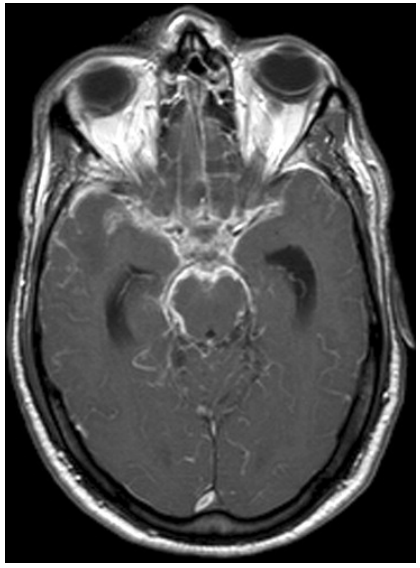

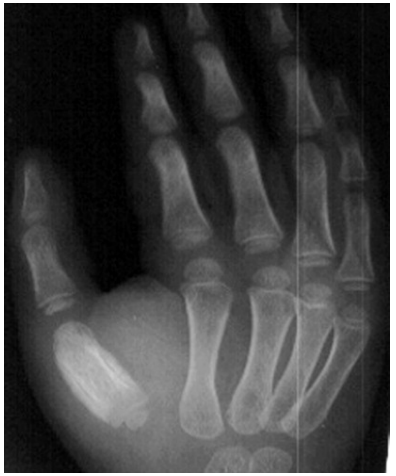

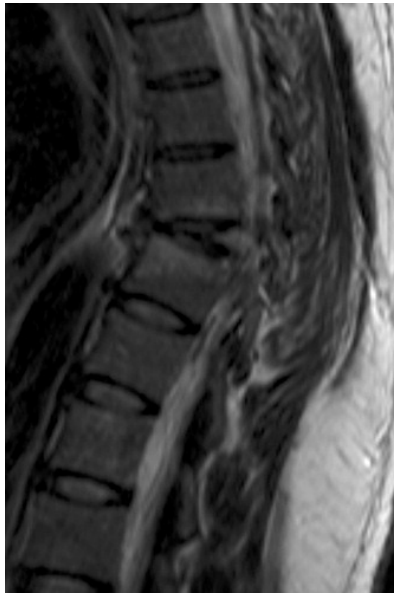

Evaluation for Active Tuberculosis

Medical History

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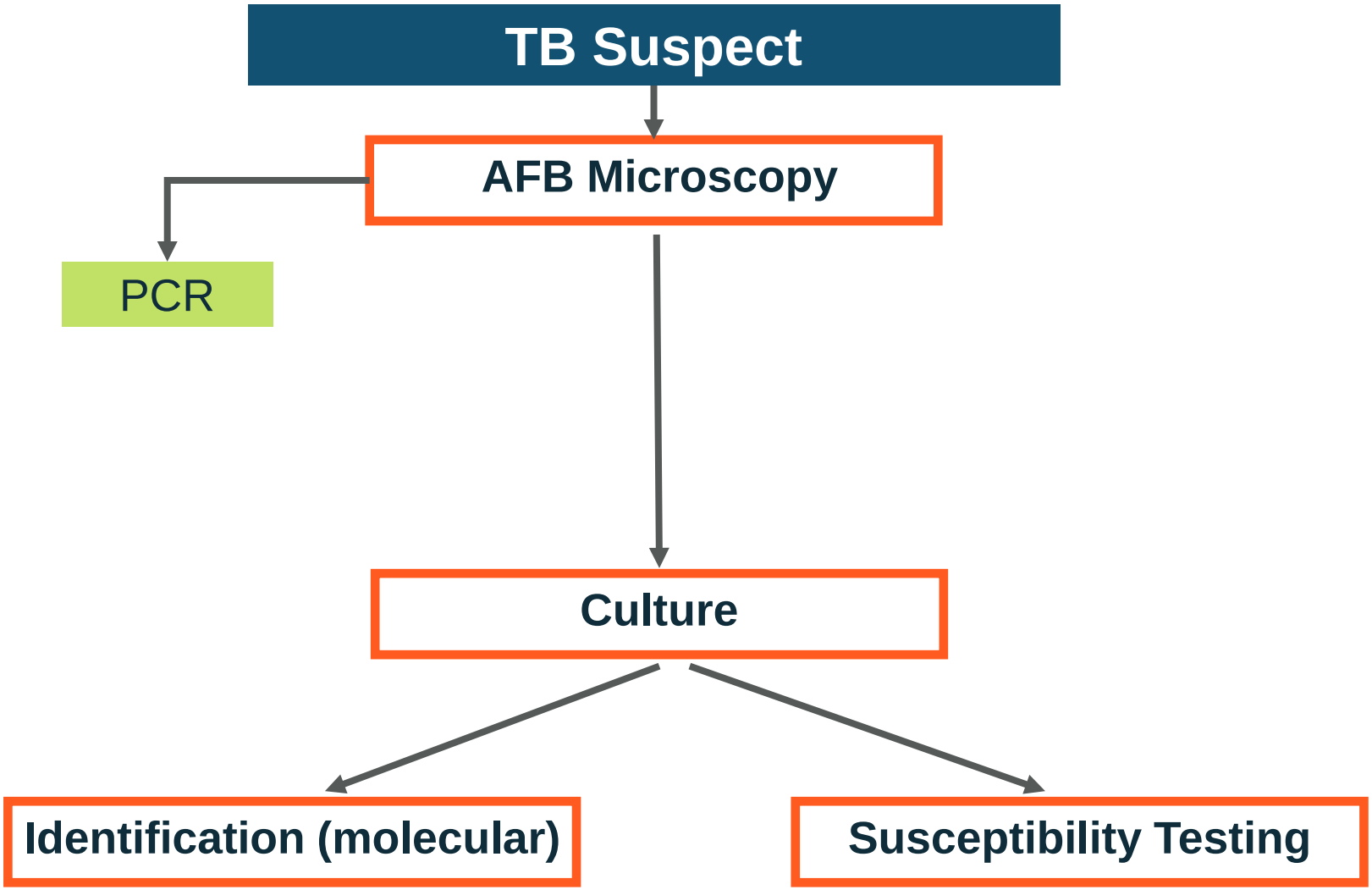
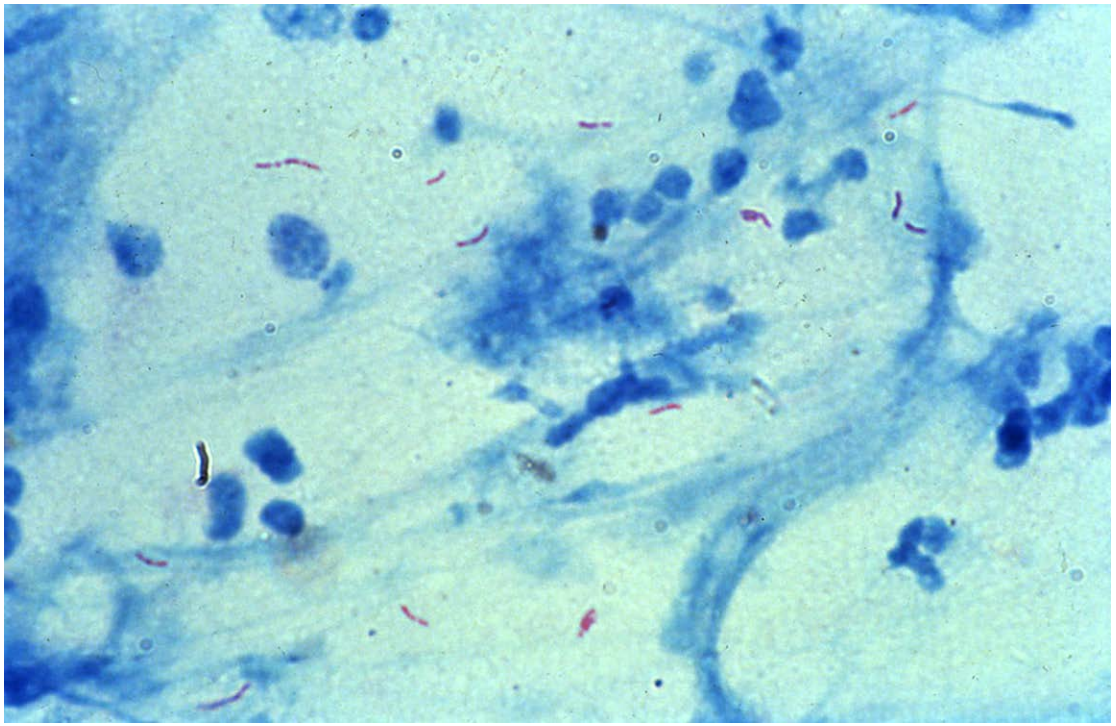
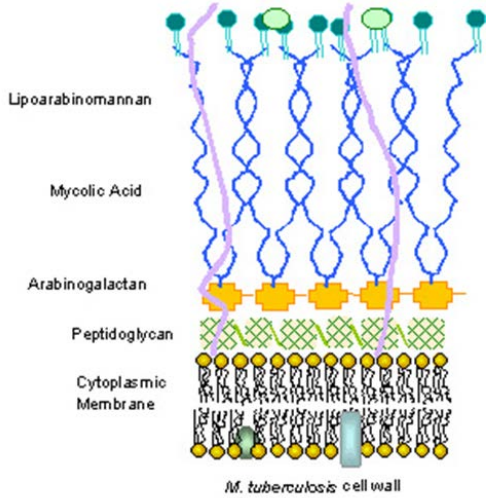
TB Diagnostic Algorithm



Stains for Mycobacteria

- Rapid - an hour to perform and report
- Inexpensive indication of whether the specimen contains mycobacteria
- Mycobacteria do not stain with the Gram stain
- “Acid-fast” stains auramine/rhodamine, Ziehl-Neelsen, or Kinyoun stain
- A complex is formed between mycolic acid and dye used in the stain
- This complex is resistant to destaining by mineral acids (thus “acid-fast”)





Acid-fast stains - Issues

- Acid-fast stains are not very specific
  - indicates whether a mycobacterium is present in the specimen
  - does not allow us to know which mycobacteria it is
    - *M. tuberculosis* looks like all the other mycobacterial species on an acid-fast stain
- Acid-fast stains are not very sensitive
  - need 1000-10,000 CFU/ml for a positive AFB smear
- Quality of sputum obtained variable

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2-3 AFB Smears are More Sensitive than 1 Smear

Yield of Serial AFB Smears

Study	% of Total Positives Detected by:		
	1st Smear	2nd Smear	3rd Smear
Walker et al. (2000), *Int J Tuberc Lung Dis*, 4:246.	77.1%	15.0%	7.9
Ipuge et al. (1996), *Trans R Soc Trop Med Hyg*, 90:258.	83.4%	12.2%	4.4%
Saleem et al. (2007) *Pak J Med Res*, 46:94-7.	66.2%	24.0%	9.8%
Mathew et al. (2002) J Clin Microbiol, 40:3482-4 (low prevalence pop.)	89.4%	5.3%	5.3%

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Acid-Fast Smears Prepared from Early Morning Sputum Specimens Have Better Sensitivity

Study	Spot (Random) Specimen Positive (%)	Early Morning Specimen Positive (%)
Ssengooba et al, 2012, *Tuberc Res Treat*, 2012: 1-6. (MGIT culture positive for MTB)	12/21 (57%)	21/21 (100%)
Abraham et al, 2012, *Indian J Med Res*, 135: 249-51 (smear is positive)	21/49 (43%)	32/49 (65%)

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Mycobacteria Cultures

Necessary to obtain an isolate of the mycobacterium for:

- species identification
- antimicrobial susceptibility testing

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Culture of *M. tuberculosis* complex

- Sensitivity of culture is much better than smear
  - a positive AF smear requires 1000-10,000 CFU/ml of specimen
  - a positive mycobacteria culture requires only 10-100 CFU/mL of specimen

Culture

- 2 types of media used:
  - Solid Medium (Lowenstein-Jensen (LJ) or Middlebrook)
  - Broth (Liquid) Medium (FDA-cleared systems - Bactec MGIT and Trek VersaTREK)
  - In general, mycobacteria grow faster in broth but there are some strains that grow better on solid medium

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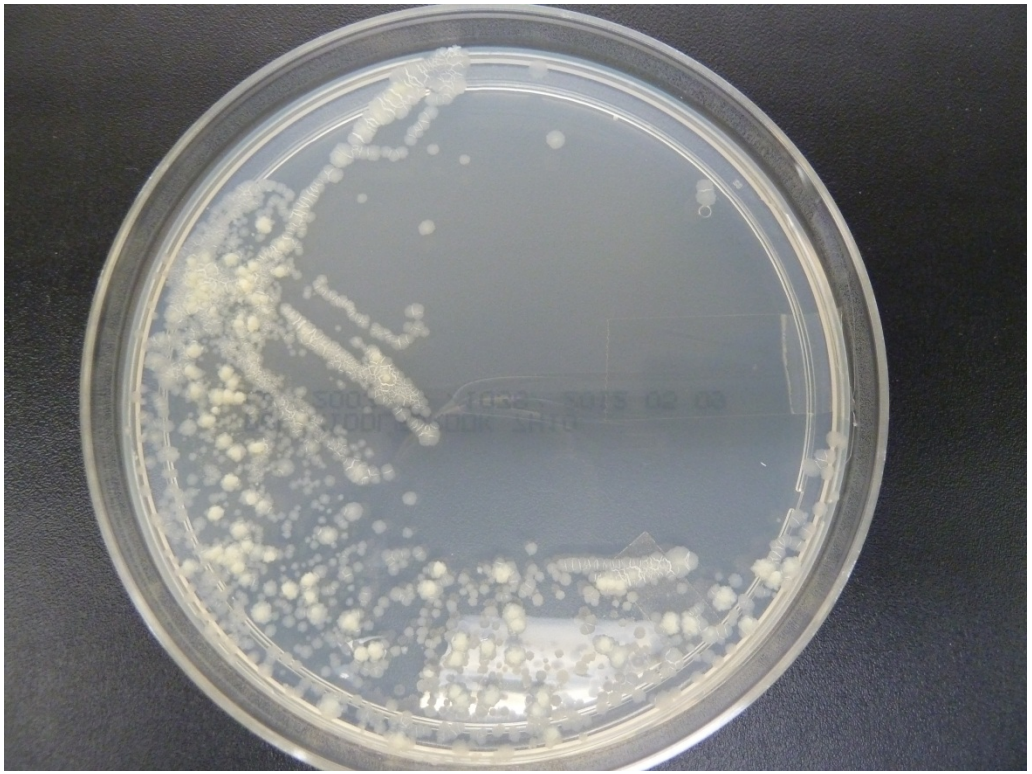
M. tuberculosis Colony Morphology on Solid Medium



Note the “rough and buff” morphology typical of *M. tuberculosis*

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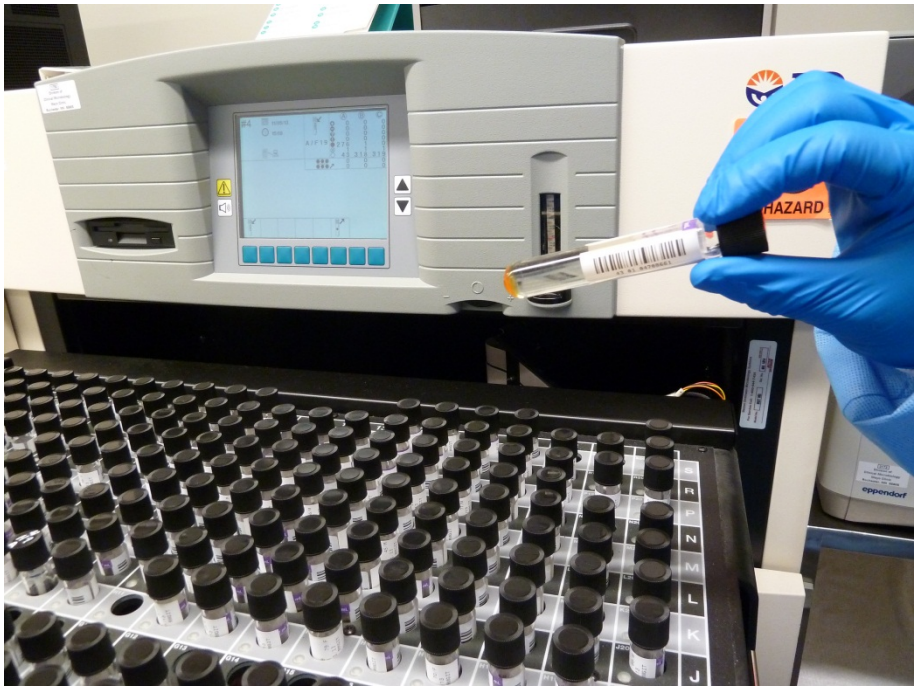
BACTEC MGIT 960 Culture System

MGIT - Mycobacterial Growth Indicator Tubes (Becton Dickinson)

- fluorescent indicator in bottom of tube quenched by O_2
- ↑ mycobacterial growth = ↓ O_2 and ↑ fluorescence



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VersaTREK System

• mycobacterial growth causes changes in bottle headspace pressure which are detected by the instrument; sponges in bottle provide increased surface area for growth

http://www.trekds.com/products/versaTREK/mdst.asp

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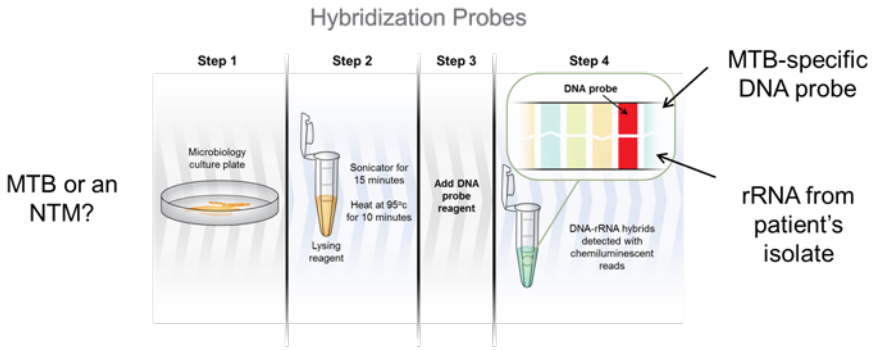
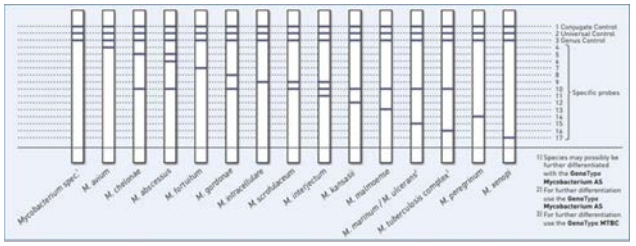
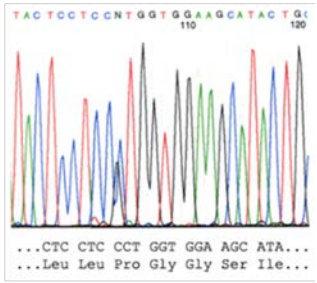
Identification of MTB from Culture Isolates



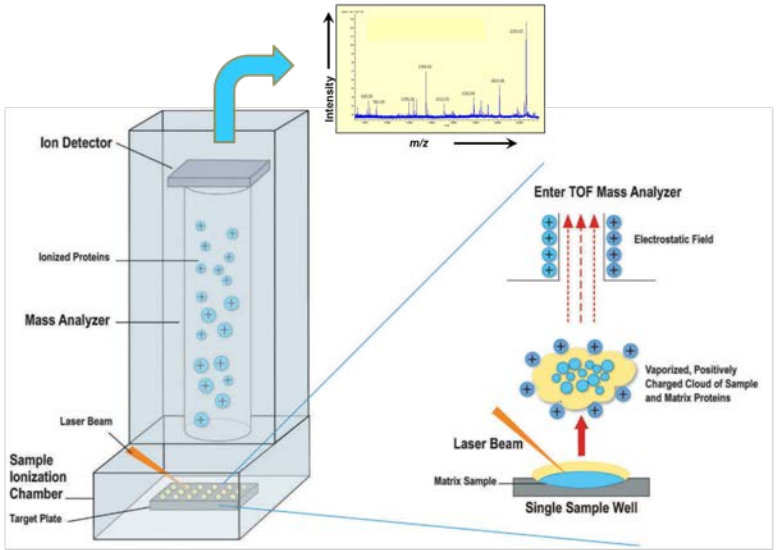
Hologic Gen-Probe AccuProbes® (nucleic acid hybridization probes)



Line Probe Hybridization Assays



DNA Sequencing



Matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry (MS)

Direct Identification of *M. tuberculosis* complex from patient specimen *without waiting* for growth in culture

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Nucleic Acid Amplification-based (NAA) tests for MTB

- CDC recommendation:
  - NAA testing be performed on at least one (preferrably the first) respiratory specimen from each patient with suspected pulmonary TB
    - if it would alter case management
    - If it would alter TB control activities
  - NAA testing does not replace the need for culture

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NAA Tests for Direct Detection of MTB

- FDA-cleared
  - Hologic/Gen-Probe MTD
  - Cepheid GeneXpert MTB/RIF
- CE-marked/RUO in U.S.
  - Hain LineProbe
- Laboratory Develop Tests (LDTs)
  - Rapid cycle/real-time PCR

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Direct Detection of MTB from Patient Specimens *Mycobacterium tuberculosis* Direct Test (MTD) (Hologic Gen-Probe)

- Transcription-mediated amplification of *M. tuberculosis* complex rRNA directly from respiratory specimens
- Clinical specificity: 99-100%
- Clinical sensitivity:
  - smear positive: 91-95%
  - smear negative: 83-100%

- Technically challenging
  - inhibition from specimen components a concern
  - open PCR system so false positives due to cross-contamination of specimens are possible.
  - cross-reactions occur w/ some rare mycobacteria: *M. celatum*, *M. terrae*-like organisms, *M. holsiaticum*

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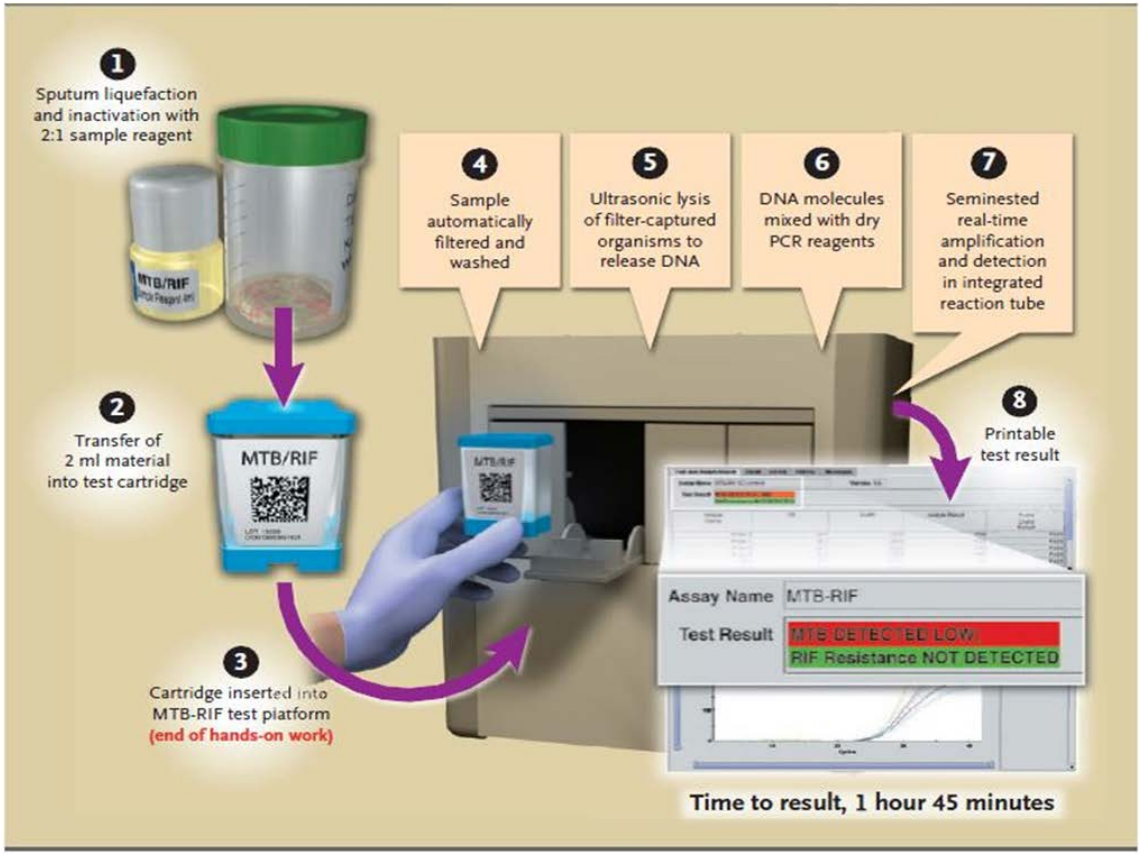
Direct Detection of MTB from Patient Specimens
Cepheid Xpert® MTB/RIF Test

- WHO-endorsed
- Runs on the Cepheid GeneXpert platform
- FDA-approved for <u>respiratory</u> specimens
- Detects *M. tuberculosis* complex and provides information about RIF resistance
- Results in about 2 hrs; minimal hands-on needed



Source: www.finddiagnostics.org

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Xpert MTB/RIF accuracy for detection of *Mtb* complex

- Limit of Detection is 131 CFU/ml (package insert)
- Chang et al, 2012, J Infect 64:580-8:
  - Meta-analysis of 18 studies with 10,224 patients total
  - Pulmonary TB:
    - Sensitivity, Smear positive disease – 98.7%
    - Sensitivity, Smear negative disease – 75.0%
    - Specificity - 98.2%
  - Extrapulmonary TB:
    - Sensitivity - smear positive, 95.2%; smear negative 70.7%
    - Specificity – 82.6%
- Time to diagnosis comparison:
  - Smear microscopy = same day (but non-specific)
  - Broth culture took an average of 16 days
  - Solid media plate cultures took an average of 20 days
  - Xpert MTB/RIF– same day diagnosis

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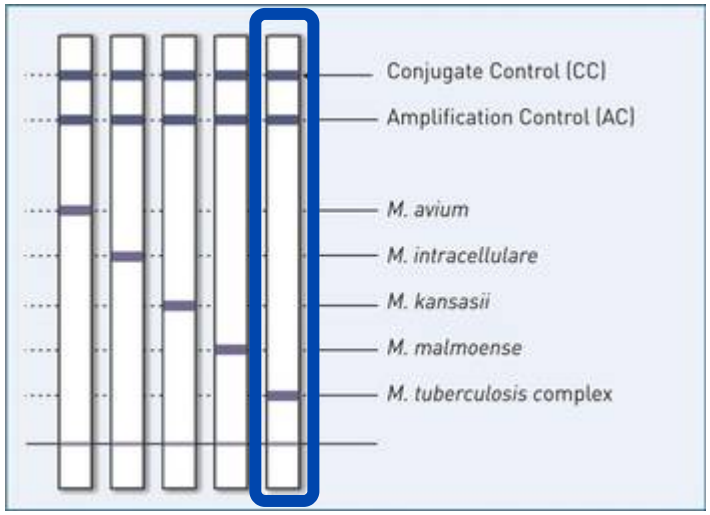
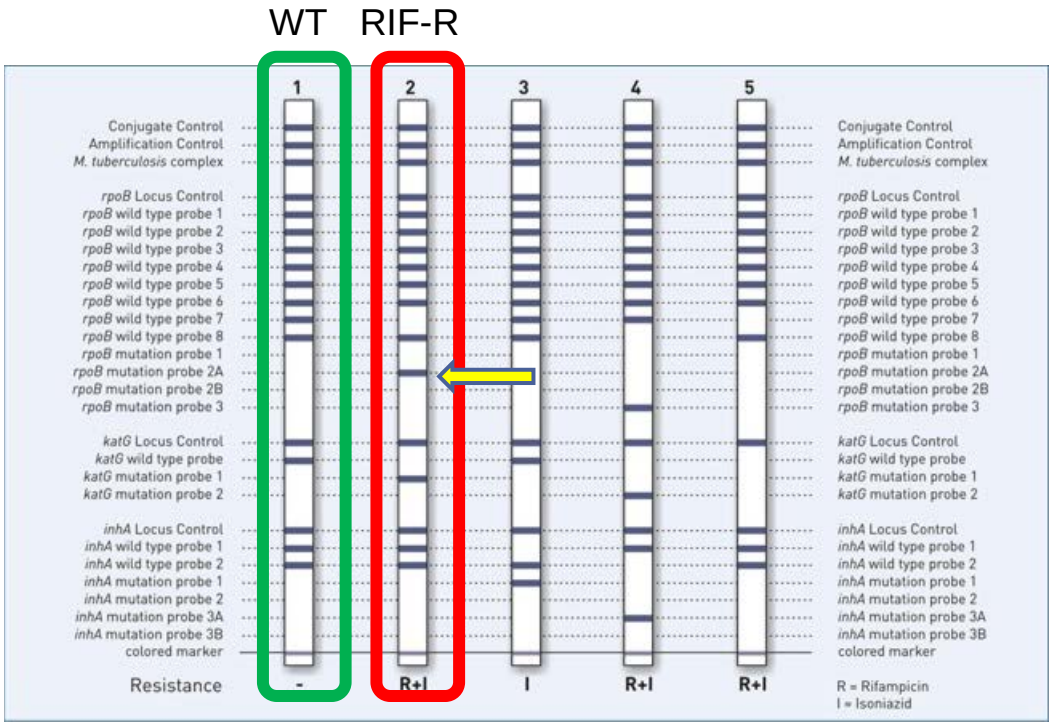
Direct Detection of MTB from Patient Specimens
Line Probe Assays
(Hain Lifesciences)



M. tuberculosis complex direct detection

Not approved for diagnostic use in the U.S.

M. tuberculosis complex detection and INH/RIF resistance



Source: http://www.hain-lifescience.de

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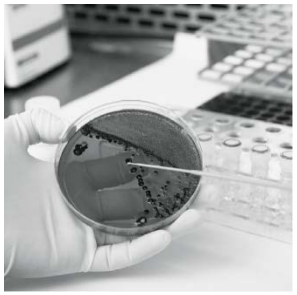
Direct Detection of MTB from Patient Specimens
Laboratory-developed PCR Tests (LDTs)

Example of Real-time PCR Workflow in our Laboratory



specimen or culture lysis, inactivation and processing

DNA extraction

PCR amplification and detection

Approximate turn-around time = 4h

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Direct comparison of Mayo LDT PCR assay with the GenProbe MTD test

Assay		MTD			
		+	-	Agreement (%)	kappa coefficient
LightCycler PCR	+	49	1	538/542 (99.3%)	0.96
	-	3	489		

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Limitations of NAA tests for Direct Detection of *M. tuberculosis*

- Inhibition from specimen components a concern for falsely negative results
  - Inhibition control needed unless system lab has checked for inhibitors in all specimen types
- PCR detects presence of nucleic acid but doesn't indicate if the organism is still viable
  - patient could be being treated successfully but may still have a positive PCR result
- Culture is more sensitive so always perform cuture too
  - negative PCR result does not rule out *M. tuberculosis* infection
  - culture isolate is needed for drug susceptibility testing

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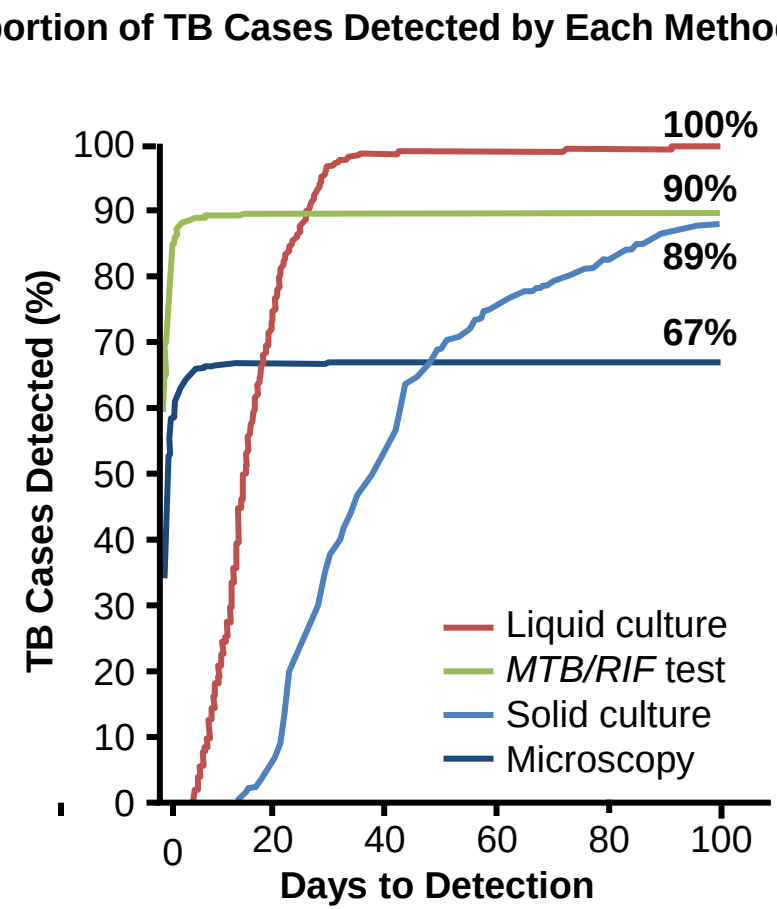
Comparison of *TB* Diagnostic Modalities



Boehme C, et al. Lancet. 2011;377:1495-1505.

Drug Resistance Testing of *M. tuberculosis* complex

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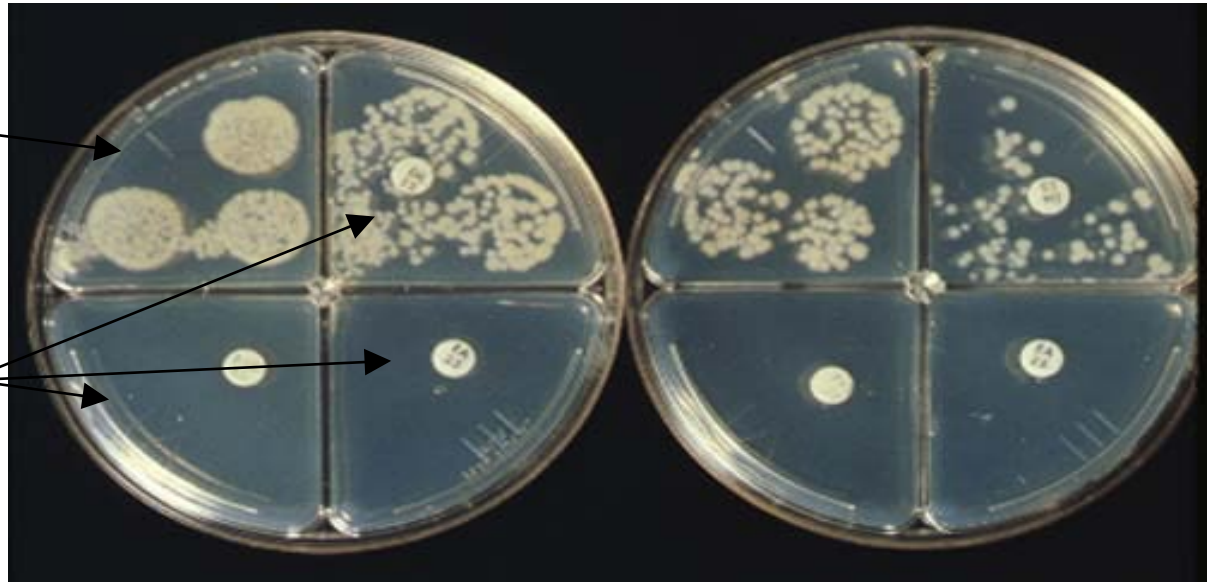
M. tuberculosis complex Drug Resistance Testing

- Agar proportion is the current gold standard for all drugs except pyrazinamide
  - not rapid (14-21 days)
  - labor-intensive, technically complex
  - no FDA-cleared, commercially-available kit



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.

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Rapid Broth Susceptibility Testing for MTB
FDA-cleared, semi-automated with MGIT or VersaTREK systems

Compare growth rates in bottles/tubes +/- critical concentrations of drug

CDC goal is results for first-line drugs reported within 15-30 days after receipt of the specimen

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M. tuberculosis complex resistant isolates

- If the isolate is resistant to any agent
  - preliminary report issued
  - consider confirming resistance by 2nd method or 2nd lab
  - consider initiating testing of secondary agents to avoid delays
- If the isolate is resistant to only PZA consider
  - speciation
    - *M. bovis* is mono-PZA-resistant
    - most isolates of *M. tuberculosis* are PZA-susceptible

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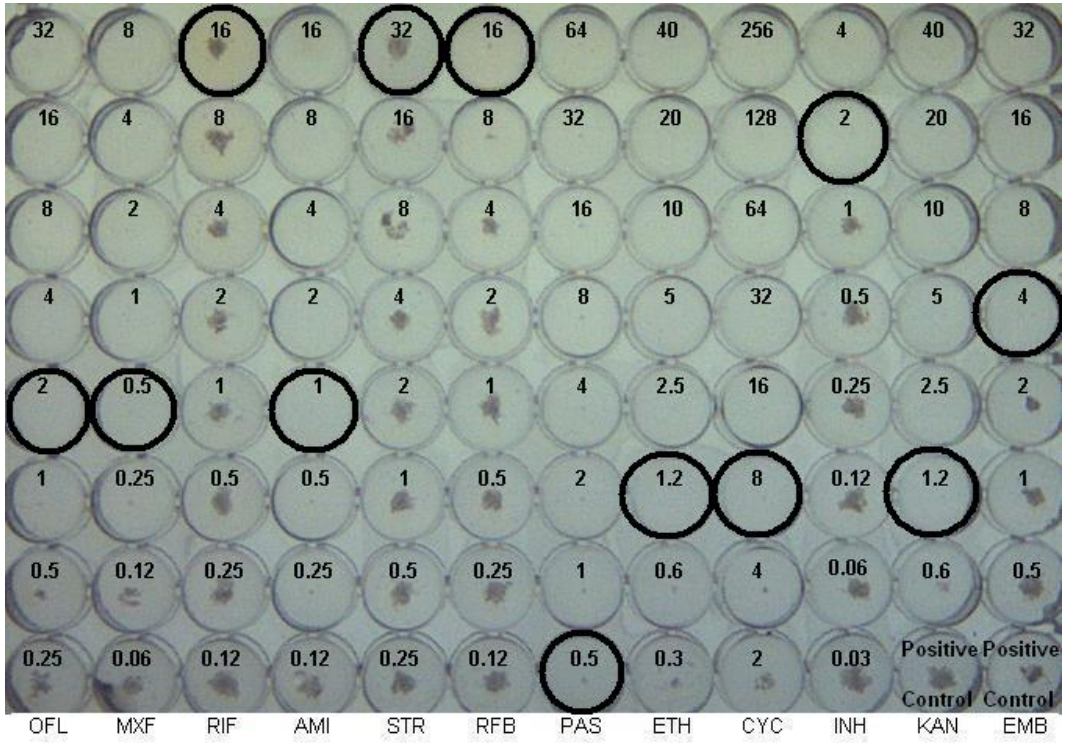
Newest Method for Mtb DST
LDT (Not FDA-cleared) MIC Plate



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.

- broth microdilution method
- multi-center studies supporting FDA-submission completed
- 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 – helpful for isolates with MIC near critical concentration (CC) breakpoint that give fluctuating results w/CC method

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Molecular detection of *Mtb* drug resistance markers

Why?

Rapid determination of potential drug resistance compared with phenotypic methods

Limited availability at this time except for the CDC MDR TB program

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Molecular Detection of *M. tuberculosis* Drug Resistance at the CDC

- Offered for *M. tuberculosis* complex isolates and nucleic-acid amplification-positive (NAAT+) sputum sediments
- Provides rapid identification of mutations associated with resistance to many TB drugs
- Limitations include
  - Insufficient data to definitively associate all mutations detected with resistance;
  - Not all mechanisms of resistance are known
  - Not all resistance loci are sequenced
- use in conjunction with conventional DST results

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Molecular resistance testing for MTB at the CDC

Drug	Locus/Loci examined	Sensitivity	Specificity
rifampin	*rpoB*	97.1	97.4
isoniazid	*inhA* & *katG*	86.0	99.1
fluoroquinolones	*gyrA*	79.0	99.6
kanamycin	*rrs* & *eis*	86.7	99.6
amikacin	*rrs*	90.0	98.4
capreomycin	*rrs* & *tlyA*	55.2	91.0
ethambutol	*embB*	78.8	94.3
pyrazinamide	*pncA*	86.0	95.9

http://www.cdc.gov/tb/topic/laboratory/MDDRUsersGuide.pdf

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Xpert MTB/RIF and Rifampin resistance

- Target is *rpoB*: gene encoding beta subunit of bacterial RNA polymerase
- Mutations in an 81bp region of the *rpoB* gene are responsible for ~96% of RIF resistance in *Mtb;*
- also predicts MDR TB since the majority of RIF-resistant isolates will also be INH-resistant
- Some false positive RIF resistance with Xpert
  - PPV is lower in low prevalence settings
  - CDC recommends reporting Xpert RIF-R as a preliminary result pending confirmation with sequencing; growth-base DST is still required

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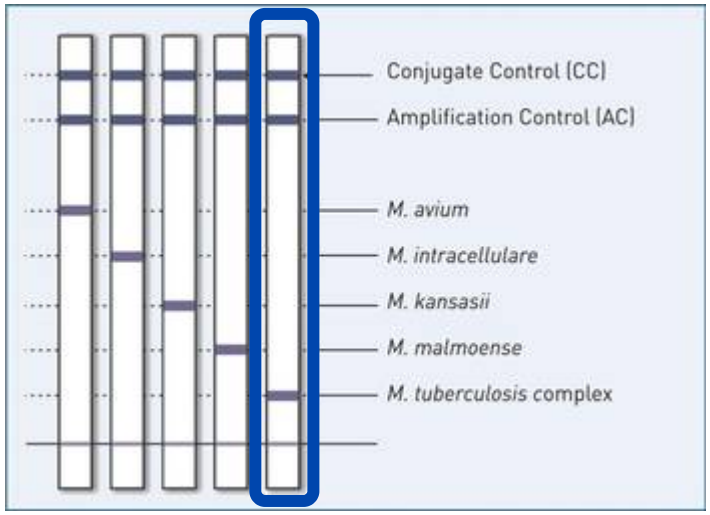
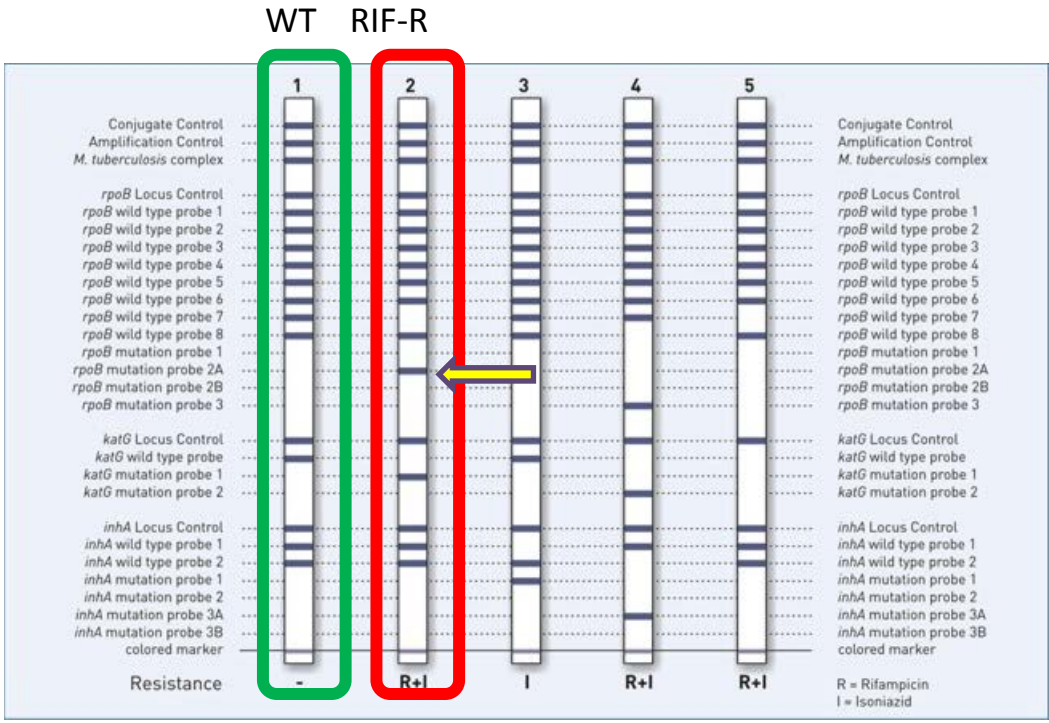
Line Probe Assays



M. tuberculosis complex direct detection

Not approved for diagnostic use in the U.S.

M. tuberculosis complex detection and INH/RIF resistance



Source: http://www.hain-lifescience.de

Diagnosis of TB: Summary

- Medical evaluation is critical
  - Identify risk of exposure and risk of reactivation
  - Clinical symptoms can be suggestive but often nonspecific
- AFB stains are rapid but insensitive and nonspecific
- Molecular tests are available for rapid identification of MTB from culture as well as from initial specimens
- Mycobacterial culture should always be ordered
- Drug susceptibility testing should be performed on all positive cultures
- Molecular tests are available for rapid identification of MTB from culture as well as from initial specimens

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