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Laboratory Diagnosis of Tuberculosis

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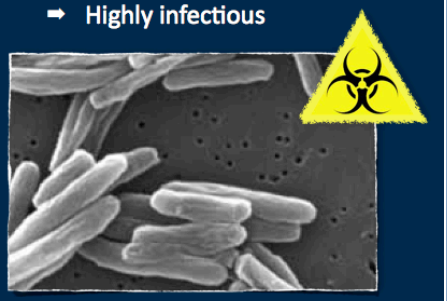
Laboratory Diagnosis of Tuberculosis
Major Challenges

- Contamination of sputum with oral bacteria
- Slow grower
- Highly infectious

10^{10-12} bacteria/ml



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Laboratory Diagnosis of Tuberculosis

Specimen collection
↓
Specimen processing → NAATs
↓
Microscopy
↓
Culture
↓
Identification
↓
Susceptibility testing

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Specimen Collection for Mycobacteriology

✓ **Sputum Quality**
- Mouth rinse with water → reduce oral flora
- Deep cough → lower respiratory secretions

✓ **Sputum Volume**
- Should exceed 5 ml

(Warren et al. *Am J Respir Crit Care* Med 2000 161:1559)

✓ **Sputum Frequency**
- 2 to 3 samples collected over 1 to 2 days

(Davis et al. Am *J Respir Crit Care Med* 2013 13:147)

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Specimen Processing for Mycobacteriology

✓ **Nonsterile samples**
- Decontaminated
- Concentrated

✓ **Sterile samples**
➡ Fluids
- Concentrated

➡ Tissue
- Homogenized

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Processing "Contaminated" Specimens

1. Decontaminate specimen with NaOH.

2. Liquify with a mucolytic agent, e.g. N-acetyl-L-cysteine

3. Centrifuge at 3000 x g.

4. Neutralize and dilute with buffer.

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AFB Specimen Processing in BSL3

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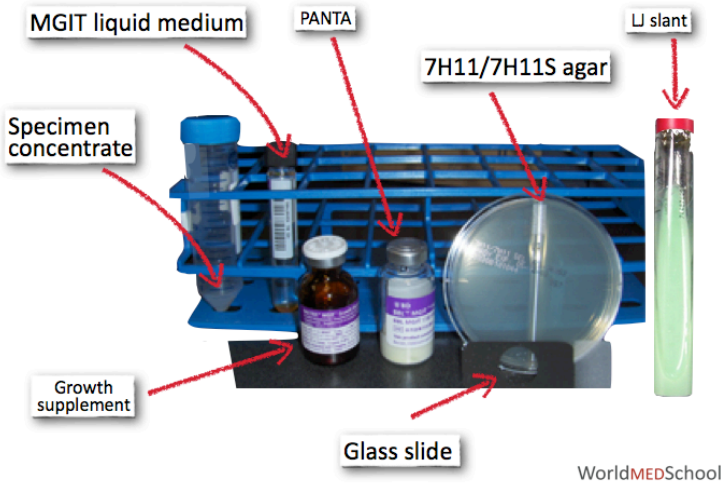
Inoculation of Media and Microscopy



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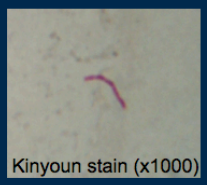
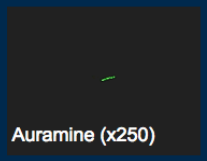
AFB Smears of Processed Specimens

- Carbol fuchin stained smear
  - ✓ Ziehl-Neelsen or Kinyoun
  - ✓ Read 300 high power fields
  - ✓ ~32-94% sensitive vs Cx



Kinyoun stain (x1000)

- Flurochrome stained smear
  - ✓ - Auramine +/- Rhodamine
  - ✓ - Read 30 low power fields
  - ✓ 52%-97%; average 10% more sensitive
  - ✓ 26-100% ↑sensitivity in HIV+
  - ✓ ~60-70% sensitive



Auramine (x250)

Steingart et al. *Lancet Infect Dis* 2006 6:664

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Reporting of Acid-Fast Smears

Report	Fuchin stain (high power)	Fluorochrome stain (low power)
Negative	0/300 F (3 sweep)	0/30 F (1 sweep)
Suspicious	1-2/300 F (3 sweep)	1-2/30 F (1 sweep)
1+	3-9/100 F (1 sweep)	3-9/10 F
2+	1-9/10 F	1-9/ F
3+	1-9/ F	10-90/ F
4+	>9/ F	>90/ F

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Factors That Limit AFB Smear Sensitivity
Overall Sensitivity 22-80%

- Bacterial load
  - 10^4 cfu/ml of specimen → 60% positive smear
- Specimen volume
- Direct vs. concentrated smears
- Staining method (Flurochrome>ZN>Kinyoun)
- Experience of reader
- Patient population (Pretreated, HI

Parsons et al *Clin Mirobiol Rev* 2011 12:314

Use of Liquid Culture and Solid Media is Recommended

✓ **Liquid Media**
- More rapid
- Automated

✓ **Solid Media**
- Mixed morphologies visible
- Contamination recoverable

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Automated Liquid Culture Systems

METHOD	TREK	BacT/ALERT	MGIT
Marker	↑Pressure	↑CO_2	↓O_2

VersaTREK

BacT/ALERT 3D

MGIT 960

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MGIT Culture System

MGIT 960

320 tubes per unit

Positive

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Solid Media Used to Culture Mycobacteria

- ✓ **Egg-based**
  1. Lowenstein-Jensen (LJ slant)
- ✓ **Agar-based**
  2. Middlebrook (7H11 plates)

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Incubation of Mycobacterial Cultures

Media	Incubator
Liquid	*37°C, 6 wks*
Solid	*CO2, 37°C, 6 wks*

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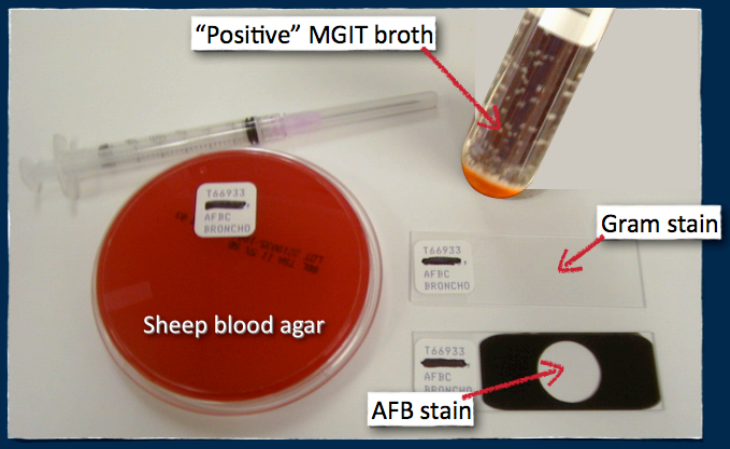
What happens when the broth turns "positive"?



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Mycobacterial Culture Identification

✓ **Phenotypic**
- Biochemical tests
- Lateral flow immunochromatographic
- High-performance liquid chromatography (HPLC)
- Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS)

✓ **Genotypic**
- Probes
  - Gen-Probe AccuProbe®
  - Line Probe
- PCR
- Sequencing

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Lateral Flow for MPB 64 protein to Identify M. tuberculosis Complex

1. Capilia TB rapid diagnostic test (Tauns Laboratories Inc., South Korea)
2. SD Bioline TB AG MPT64 Rapid test (Standard Diagnostics, South Korea)
3. BD MGIT TBc identification test (BD Diagnostics, Sparks, MD)

Overall sensitivity of 98.6% and specificity of 97.9%
Parsons et al *Clin Mirobiol Rev* 2011 12:314

Mycobacterial Culture Identification

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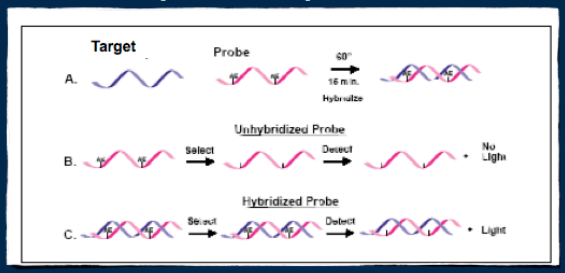
Commercial Identification Methods

AccuProbe (Gen-Probe)



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Line Probe Assays

- Nitrocellulose strip
- Specific probes attached as parallel lines
- Requires a PCR amplification step
- Visual detection or automated reader

- INNO-LiPA MYCOBACTERIA v2 (Innogenetics)
- GenoType® Mycobacterium CM/AS (HAIN)

http://www.hain-lifescience.html
http://www.innogenetics.com/

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Drug Susceptibility Testing for M. tuberculosis

First-line drugs	Second-line drugs
Isoniazid	Amikacin
Rifampin	Capreomycin
Ethambutol	Ethinamide
Pyrazinamide	Ethambutol
	Kanamycin
	Linazolid
	Quinolone
	p-Aminosalicylic acid
	Rifabutin
	Streptomycin

- First isolate
- Retest if fail to respond

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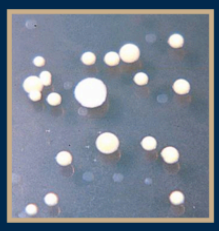
Drug Susceptibility Testing Methods

Solid Media



VersaTREK

MGIT 960

1. Absolute conc.
2. Proportion method
3. Resistance ratio

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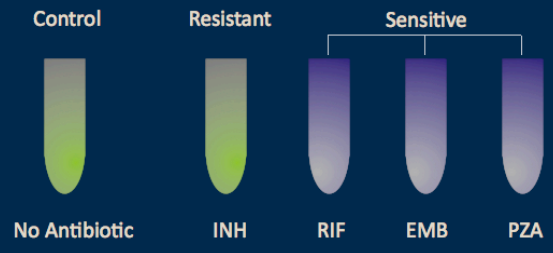
MGIT960 Drug Susceptibility Assay



TAT: 4-13 days
<4 or >13 days: No result

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Nucleic Acid Amplification Assays for Direct Detection of *M. tuberculosis* in Specimens

1. MDT Direct Test *, Gen-Probe Inc
   Method: TMA
2. COBAS AMPLICOR *; COBAS TaqMan MTB, Roche
   Method: PCR ELISA; Real-Time PCR
3. GeneXpert MTB/RIF #, Cepheid
   Method: Real-Time PCR
4. GenoType MTBDRplus #, HAIN
   Method: Line Probe
5. Homebrewed PCR assays

* US FDA approved
\# WHO endorsed

Performance of NAATs for Detection of *M. tuberculosis* in Clinical Specimens

Direct NAA assay	Company	Method	% sensitivity (range)		
			Smear-positive samples	Smear-negative samples	All samples
Amplicor PCR	Roche Molecular Systems	PCR	90–100	50–95.9	91.3–100 (overall)
Amplified MTD	Gen-Probe Inc.	Transcription-mediated amplification	91.7–100	65.5–92.9	92.1–100 (overall)
BD ProbeTec	Becton Dickinson	Strand displacement amplification	98.5–100	33.3–85.7	98.9–100 (overall)
GenoType Mycobacteria Direct	Hain Lifescience	Transcription-mediated amplification	85.7–94.6	33.3–65.4	99.6–100
LAMP	Eiken Chemical Co.	Loop-mediated isothermal amplification	97.7	48.8	99 (overall)

Parsons et al *Clin Mirobiol Rev* 2011 12:314

Direct Genotypic Susceptibility Testing

Drug	Targets	Sensitivity
Rifampin	rpoB	98%
INH	(katG), inhA	85%

Lin et al. *J Clin Microbiol* 2004 42:4204

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Thank you!

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