“TB as a cause of the right upper lobe pneumonia in this 23 y/o male from Somalia was ruled out with 3 negative sputum smears. The patient was released from isolation and was discharged home to complete an outpatient course of levofloxacin and to follow-up with primary care.”

Anonymous
# Missed TB Diagnoses

California, 2005-2011

## Table 2. Counts and Prevalence of Potential Misdiagnoses for Various Potential Misdiagnosis Window

<table>
<thead>
<tr>
<th>Potential Misdiagnosis Window</th>
<th>TB Cases With Previous Visit and Respiratory Diagnosis</th>
<th>TB Cases With Previous Visit and No Respiratory Diagnosis</th>
<th>Potential Misdiagnosis Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-30</td>
<td>513</td>
<td>261</td>
<td>15.9%</td>
</tr>
<tr>
<td>5-60</td>
<td>714</td>
<td>426</td>
<td>22.2%</td>
</tr>
<tr>
<td>5-90</td>
<td>826</td>
<td>528</td>
<td>25.7%</td>
</tr>
<tr>
<td>5-120</td>
<td>880</td>
<td>609</td>
<td>27.3%</td>
</tr>
<tr>
<td>5-180</td>
<td>953</td>
<td>741</td>
<td>29.6%</td>
</tr>
<tr>
<td>5-270</td>
<td>1027</td>
<td>871</td>
<td>31.9%</td>
</tr>
<tr>
<td>5-360</td>
<td>1078</td>
<td>963</td>
<td>33.5%</td>
</tr>
</tbody>
</table>

Abbreviations: TB, tuberculosis.

* Proportion of TB patients (of 3220 patients in the final sample) having a previous visit with a respiratory diagnosis occurring in a given potential-misdiagnosis window.

Which of the following statements is false about diagnosis of pulmonary tuberculosis?

A. The combination of cough, sputum and fever-or-weight loss is up to 90% sensitive but <50% specific.

B. The predictive value of a negative TST in a patient with a clinical presentation highly suggestive of severe TB could be as low as 50%.

C. >95% of active pulmonary TB cases report cough.
Learning Objectives

• Recognize clinical syndromes warranting evaluation for active TB.
• List the elements of a complete evaluation for active TB.
• Upon completion of an initial evaluation, conduct medical decision making regarding initiation of therapy.
Active TB Evaluation

• History and Examination
• Imaging
• Mycobacteriology
• Ancillary tests
• Specialty evaluation
Initial Clinical Evaluation

• Symptoms
• Prior TB diagnosis/treatment
• Epidemiologic risk
• Predisposing medical conditions/therapies
• General, temperature, weight
• Lymphadenopathy
• Chest auscultation abnormalities
• Abdominal distension
• Neurologic abnormalities
### Clinical Presentation: Signs and symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough (dry/productive sputum)</td>
<td>75-80%</td>
</tr>
<tr>
<td>Weight loss</td>
<td>45-75%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>60-70%</td>
</tr>
<tr>
<td>Fever</td>
<td>50-60%</td>
</tr>
<tr>
<td>Night Sweats</td>
<td>50-55%</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>25-35%</td>
</tr>
<tr>
<td>No symptoms</td>
<td>10-20%</td>
</tr>
</tbody>
</table>

*Source: Barnes 1988, Miller 2000*
Accuracy of Symptom Check

<table>
<thead>
<tr>
<th></th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough (any)</td>
<td>93%</td>
<td>37%</td>
<td>21%</td>
<td>97%</td>
</tr>
<tr>
<td>Night sweats (24h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever (2wks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia (4wks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Good news: effective
Bad news: inefficient

Clinical Presentation: Site of Disease

CDC Reported TB Cases by Form of Disease United States, 2010

- Pulmonary (68%)
- Lymphatic (40%)
- Extrapulmonary (22%)
- Pleural (16%)
- Other (18%)
- Bone/joint (10%)
- Genitourinary (5%)
- Peritoneal (5%)
- Meningeal (6%)
- Both (10%)
- Meningeal (6%)
Epidemiologic Risk Factors for TB

- Known contact to a pulmonary case
- Nation of origin
- Extended stays in high-risk areas
- Health care work *in certain settings*
- Incarceration *in certain settings*
- Homelessness
- Other occupational exposures
Relative risk of TB reactivation by medical condition

<table>
<thead>
<tr>
<th>Condition</th>
<th>Study</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced HIV infection</td>
<td>Pablos-Mendez et al. 27, Moss et al. 26</td>
<td>9.9 (8.7–11.3)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.4 (3.5–25.1)</td>
</tr>
<tr>
<td>Old, healed tuberculosis</td>
<td>Ferebee, 13 Ferebee et al. 20</td>
<td>5.2 (3.4–8.0)</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>Pablos-Mendez et al. 27</td>
<td>2.4 (2.1–2.8)†</td>
</tr>
<tr>
<td>Infliximab therapy</td>
<td>Keane et al. 28</td>
<td>2.0 (0.7–5.5)†</td>
</tr>
<tr>
<td>Poorly controlled diabetes</td>
<td>Pablos-Mendez et al. 27</td>
<td>1.7 (1.5–2.2)†</td>
</tr>
<tr>
<td>Silicosis</td>
<td>Cowie 29</td>
<td>1.7 (1.3–2.1)†</td>
</tr>
<tr>
<td></td>
<td>Corbett et al. 30</td>
<td>1.3 (1.1–1.7)†</td>
</tr>
<tr>
<td></td>
<td>Kleinschmidt and Churchyard 31</td>
<td>1.2 (1.0–1.5)†</td>
</tr>
<tr>
<td>Underweight (≤10 percent below normal)</td>
<td>Palmer et al., 22 Edwards et al. 23</td>
<td>1.6 (1.1–2.2)</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>Thorn et al. 32, Steiger et al. 33</td>
<td>1.4 (1.1–1.9)†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 (1.2–1.4)†</td>
</tr>
</tbody>
</table>

Horsburgh, NEJM 2004 350; 20: 2060-7
Differential Diagnosis

- Community acquired pneumonia
- Malignancy
- Lung abscess
- Non-TB mycobacteria
- Fungal infection
- Parasite (e.g., paragonimiasis)
- Sarcoidosis
- Rheumatologic disease (e.g., Wegener’s, RA)
- Other systemic infections (e.g. brucellosis, melioidosis, relapsing fever, etc.)
Radiographic Evaluation
<table>
<thead>
<tr>
<th>TB Pattern</th>
<th>“Typical”/ Reactivation</th>
<th>“Atypical“/ Primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrate</td>
<td>85% upper</td>
<td>Upper:Lower 60:40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Usually upper in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>children</td>
</tr>
<tr>
<td>Cavitation</td>
<td>Often present</td>
<td>Rare</td>
</tr>
<tr>
<td>Adenopathy</td>
<td>Rare</td>
<td>Children common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults ~30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unilateral &gt; bilateral</td>
</tr>
<tr>
<td>Effusion</td>
<td>May be present</td>
<td>May be present</td>
</tr>
<tr>
<td>HIV</td>
<td>CD4 &gt;200</td>
<td>CD4 &lt;200</td>
</tr>
</tbody>
</table>
TB Symptoms with Compatible CXR

Sputum Exam
AFB smear/culture x3
TB PCR x 1-2

- **PCR positive**
  - TB Treatment
  - Public Health

- **Smear positive/PCR negative**
  - Pulmonary referral?

- **Smear/PCR Negative**
  - Observe?
  - TB Rx?
  - Rx other?
Clinical Specimens for Examination

- Sputum (x3)
- Bronchoalveolar lavage, washings, brushings
- Transbronchial biopsy
- Fine needle or core needle aspirate
- Tissue biopsy or excision (save some unfixed)
- Fluids
  - Cerebrospinal
  - Pleural fluid
  - Peritoneal fluid
  - Abscess
Collection of Respiratory Specimens

- Sputum Expectoration:
  - 3 specimens (at least 8 hours apart)
  - 1 spot specimen
  - 2 consecutive first-morning specimens

- Post-bronchoscopy sputum
Bronchoscopy Indications

- Unable to obtain specimen via induction or gastric aspirate
- Sputum smear/PCR negative but clinical suspicion of TB still high
- Sputum smear negative and MDR is a high concern
- Malignancy is suspected
Post-Bronchoscropy Sputum

- 57 sputum smear-negative or non-productive\textsuperscript{1}
  - 33% AFB smear-positive PBS
  - 7% PBS sole culture-positive specimen
- 56 culture-confirmed cases with negative sputum AFB smears or non-productive\textsuperscript{2}
  - AFB smear sensitivity:
    - BAL 57%
    - PBS 77%
    - BAL + PBS 84%
Laboratory Evaluation
Acid Fast Staining

- **Preparation**
  - Centrifugation to concentrate
  - NaOH, NaOCL wash to decontaminate

- **Ziehl-Neelsen**
  - Carbolfuchsin $\rightarrow$ acid alcohol $\rightarrow$ methylene blue

- **Flourescent**
  - Auramine-Rhodamine
Acid Fast Staining
AFB Smear Performance

AFB Culture

- Broth (faster, more expensive, complex)
  - Liberation of $^{14}$CO2 (defunct)
  - Emission of fluorescence (MGIT)
- Plates (slower, less expensive, “simpler”)
  - Lowenstein-Jensen slant
  - 7H11 plates
- Identification of AFB growth
  - Phenotypic characteristics
  - Nucleic acid hybridization
  - DNA sequencing
  - HPLC
MGIT
## AFB Culture Performance

### Table 1. MODS sensitivity and time to detection of MTB versus standard culture methods

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of samples positive by method</th>
<th>No. of samples positive by at least one culture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensitivity (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Median detection time (range)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auramine O stain</td>
<td>76</td>
<td>98</td>
<td>78</td>
<td>9 (4–31)</td>
</tr>
<tr>
<td>MODS</td>
<td>89</td>
<td>97</td>
<td>92</td>
<td>10 (4–39)</td>
</tr>
<tr>
<td>MGIT</td>
<td>88</td>
<td>95</td>
<td>93</td>
<td>10 (4–39)</td>
</tr>
<tr>
<td>LJ</td>
<td>73</td>
<td>96</td>
<td>76</td>
<td>24 (6–59)</td>
</tr>
<tr>
<td>microagar 7H11</td>
<td>75</td>
<td>96</td>
<td>78</td>
<td>14.5 (4–28)</td>
</tr>
<tr>
<td>PCR</td>
<td>81</td>
<td>90</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>
AFB Culture Limitations

• False Positive (up to 3% of total)
  – Laboratory cross contamination
  – Specimen mis-handling

• False Negative
  – Small inoculum
  – Delay in inoculation
  – Difficult-to-grow strain
# Mycobacteriologic Examinations for TB

<table>
<thead>
<tr>
<th>Test</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB stain</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td>AFB culture</td>
<td>2-6 weeks</td>
</tr>
<tr>
<td>Phenotypic DST results</td>
<td>4-8 weeks</td>
</tr>
<tr>
<td>DNA fingerprinting</td>
<td>Months</td>
</tr>
</tbody>
</table>
Culture-negative TB Diagnostic Criteria

- Compatible clinical and radiographic syndrome
- AFB cultures negative
  - 10-15% pulmonary
  - 25-30% extrapulmonary
- Clinical/radiographic improvement on therapy
- Other causes reasonably excluded
- Positive TST-or-IGRA
Presumptive Treatment for Smear-Negative Pulmonary TB

6 (8%) of 72 inactive cases with regimen altering adverse effects

# Mycobacteriologic Examinations for TB

<table>
<thead>
<tr>
<th>Test</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB stain</td>
<td>&lt;24 hours</td>
</tr>
<tr>
<td><strong>Nucleic acid amplification</strong></td>
<td>Hours-days</td>
</tr>
<tr>
<td><strong>Molecular DST results</strong></td>
<td>Days-weeks</td>
</tr>
<tr>
<td>AFB culture</td>
<td>2-6 weeks</td>
</tr>
<tr>
<td>Phenotypic DST results</td>
<td>4-8 weeks</td>
</tr>
<tr>
<td>DNA fingerprinting</td>
<td>Months</td>
</tr>
</tbody>
</table>
**Nucleic Acid Amplification Tests (NAAT)**

- **Varieties**
  - Amplified MTD (GenProbe)
  - GeneXpert Mtb/RIF (Cepheid)
  - Non FDA Approved
    - MTBDR (Hain)
    - Others
  - Laboratory developed

- **Use**
  - Directly on processed specimen
  - No current TB rx >7 days
  - No prior TB rx within past 12 months
1. Sample automatically filtered and washed
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into...
4. Ultrasonic lysis of filter-captured organisms to release DNA
5. DNA molecules mixed with dry PCR reagents
6. Seminested real-time amplification and detection in integrated reaction tube
7. printable test result

- Assay Name: MTB-RIF
- Test Result: MTB DETECTED LOW.
  - RIF Resistance NOT DETECTED
Sensitivity of Xpert in Pulm TB Dx Low & High Burden Settings

<table>
<thead>
<tr>
<th>TEST</th>
<th>SENS</th>
<th>SPEC</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert x 1</td>
<td>81.4%</td>
<td>98.7%</td>
<td>94.6%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Smear pos</td>
<td>98.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear neg</td>
<td>54.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xpert x 2</td>
<td>95.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear pos</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smear neg</td>
<td>71.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- N = 992 (US, Brazil, S Africa)
- Sensitivity of AFB smear: 60%
- No difference between high and low prevalence settings

_Leutkemeyer AF, et al. CID 2016_
Caution with NAAT in Previously Treated Patients

Theron, et al. CID 2016
- 45/321 (14%) positive were culture negative
- Recency of prior treatment
- Low DNA
- CXR not suggestive of TB

Boyles, et al. IJTLID 2014
- 4 false positive case reports
- 1, 2, 5 and 66 months after prior treatment
Mycobacteriology Flow

Specimen

AFB smear/stain

NAAT

MGIT broth and 7H10 plate cultivation

Species identification

*M. tuberculosis* complex

*M. avium* complex

*M. gordonae* complex

Other MOTT

Broth sensitivities for HRES + Z

If resistance: plate confirmation/proportional method and key second line drugs
Other Laboratory Examination

- Fluids: chemistry, cell count and cytology
- Tissues: routine histopathology → necrotizing granulomata
- Tuberculin skin test
- Interferon gamma release assays
- Adenosine deaminase
Granulomatous Inflammation
Typical Findings Extrapulmonary Specimens

- Protein elevated
  - Pleural/peritoneal (>4-5gm/dL)
  - CSF (>100-500mg/dL)
- Moderately decreased glucose (~40-50mg/dL)
- Pleocytosis
  - Pleural (1,000-5,000 WBC/uL)
  - CSF (100-500/uL)
- Lymphocyte predominant differential
- Necrotizing granulomata
- NAAT 50-75% sensitive
- AFB smear: 10-50% sensitive
- AFB culture: 60-90% sensitive
Pleural Effusion Evaluation Sensitivity

<table>
<thead>
<tr>
<th>Specimen Cultured</th>
<th>AFB Culture Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum only</td>
<td>48%</td>
</tr>
<tr>
<td>Fluid only</td>
<td>63%</td>
</tr>
<tr>
<td>Sputum + Fluid</td>
<td>79%</td>
</tr>
</tbody>
</table>


Pleural Biopsy

- Closed
  - Up to 40% of specimens contain no pleural tissue
  - Image guided gaining favor
  - Sensitivity (pathology + culture): 80-90%
- Thoracoscopy/VATS: sensitivity approaches 100%

### TST & IGRA Performance

<table>
<thead>
<tr>
<th>TEST</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RANGE</td>
<td>MEDIAN</td>
</tr>
<tr>
<td>TST</td>
<td>53-95</td>
<td>82</td>
</tr>
<tr>
<td>QFT</td>
<td>56-93</td>
<td>85</td>
</tr>
<tr>
<td>TSPOT</td>
<td>58-100</td>
<td>86</td>
</tr>
</tbody>
</table>

*Mazurek GH, et. al. MMWR 2010; 59(RR05);1-25*

**Specificity of TST in BCG vaccinated children**
- <1 y/o at vaccination: 91%
- >1 y/o at vaccination: 58%


**Sensitivity of TST in miliary vs LN TB**
- 22% vs. 73%

## Indeterminate QFT Results in Sick Patients

<table>
<thead>
<tr>
<th>Month</th>
<th>Volume</th>
<th>Inpt</th>
<th>Intp %</th>
<th>Pos</th>
<th>Neg</th>
<th>Indet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2010-Jan 2011</td>
<td>342</td>
<td>66</td>
<td>19.3</td>
<td>10.8</td>
<td>81.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Feb 2011-Mar 2011</td>
<td>455</td>
<td>60</td>
<td>13.2</td>
<td>12.7</td>
<td>76.0</td>
<td>11.4</td>
</tr>
<tr>
<td>April 2011-May 2011</td>
<td>540</td>
<td>66</td>
<td>12.1</td>
<td>14.3</td>
<td>79.6</td>
<td>6.1</td>
</tr>
<tr>
<td>June 2011-July 2011</td>
<td>565</td>
<td>62</td>
<td>11.0</td>
<td>10.4</td>
<td>83.2</td>
<td>6.4</td>
</tr>
<tr>
<td>Aug 2011-Sep 2011</td>
<td>643</td>
<td>54</td>
<td>8.4</td>
<td>12.4</td>
<td>85.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Oct 2011-Nov 2011</td>
<td>632</td>
<td>45</td>
<td>7.1</td>
<td>14.6</td>
<td>79.7</td>
<td>5.7</td>
</tr>
<tr>
<td>Dec 2011-Jan 2012</td>
<td>740</td>
<td>66</td>
<td>8.9</td>
<td>10.8</td>
<td>83.6</td>
<td>5.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Month</th>
<th>% Indets from Inpt. Sites</th>
<th>% Inpt. Samples with Indet. Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2010-Jan 2011</td>
<td>28.6</td>
<td>30.3</td>
</tr>
<tr>
<td>Feb 2011-Mar 2011</td>
<td>57.7</td>
<td>50.0</td>
</tr>
<tr>
<td>April 2011-May 2011</td>
<td>48.5</td>
<td>24.2</td>
</tr>
<tr>
<td>June 2011-July 2011</td>
<td>44.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Aug 2011-Sep 2011</td>
<td>41.7</td>
<td>18.5</td>
</tr>
<tr>
<td>Oct 2011-Nov 2011</td>
<td>36.1</td>
<td>28.9</td>
</tr>
<tr>
<td>Dec 2011-Jan 2012</td>
<td>22.0</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Koelle D. IGRA News. UW Lab Medicine, 09 Feb 2012.
Pleural Fluid ADA
Low Incidence Setting

• N=338 patients
• Lymphocytic exudative
• 7 pleural TB cases
• Typical cut-off: >40
• Sensitivity: 85%
• Specificity: 90%
• PPV: 85%
• NPV: 99%

ADA Limitations

• False negatives
  – Early disease
  – Advanced age
  – Smokers

• False positives
  – Non-TB empyema, parapneumonic effusions
  – Mesothelioma, lung and hematologic malignancies
  – Rheumatologic conditions

"We conclude that commercial IGRA's, performed either on whole-blood or pleural fluid samples, have poor diagnostic accuracy in patients suspected to have TPE."

CSF IGRA for TB Meningitis

- Meta-analysis
- 6 CSF IGRA studies included

- Sensitivity: 77%
- Specificity: 88%

Question

Which of the following statements is false about diagnosis of pulmonary tuberculosis?

A. The combination of cough, sputum and fever-or-weight loss is up to 90% sensitive but <50% specific.

B. The predictive value of a negative TST in a patient with a clinical presentation highly suggestive of severe TB could be as low as 50%

C. >95% of active pulmonary TB cases report cough
Medical Decision Making

Treat for TB or Not?
Smear positive for AFB
\[\downarrow\]
Culture and Speciation

- M. tuberculosis
  - 50-90%
- Non-tuberculous mycobacteria
  - 10-50%

Predictive value of a positive smear is reduced in populations with increased prevalence of non-tuberculous mycobacterial infection
Medical Decision Making Tool--2

Patient with **smear-positive** specimen

- **Positive NAAT**
  - MTB ≥97%
  - NTM <3%

- **Negative NAAT**
  - MTB 1-8%
  - NTM 92-99%

- 2009 CDC Guidelines: Test all AFB+/NAAT- specimens for inhibitors
- Probably not necessary if using Xpert, which tests for PCR inhibitors
Medical Decision Making Tool--3
Sputum Smear Negative

High Clinical Suspicion (e.g., 50% pre-test probability)

Perform NAAT

Positive NAAT
- MTB ≥90%
- Not MTB 10%

Negative NAAT
- MTB 25%
- Not TB 75%

• 2009 CDC Guidelines: Consider repeat test for confirmation if AFB-, NAAT+
• May not be necessary if using Xpert, given high specificity and low risk of cross-contamination
Medical Decision Making Tool--4
Sputum Smear Negatives

Low Clinical Suspicion; e.g., 5% pre-test probability

Perform NAAT??

Positive NAAT
- MTB: 20%
- Not MTB: 80%

Negative NAAT
- MTB: <1%
- Not MTB: 99%

- 2009 CDC Guidelines: Avoid NAAT in this clinical scenario
- Same holds true for Xpert, which provides no added value over smear
Drug Susceptibility Testing
Culture-Based Drug Susceptibility Testing

• MGIT broth (2-3 weeks)
  – Control, INH, RIF, EMB, STR, PZA
  – Qualitative

• Agar plates (4-6 weeks)
  – Quantitative: concentration, % resistant
  – First and second line drugs
Molecular Drug Susceptibility Testing  
Detection of Common Resistance Conferring Mutations

- Requirements: sufficient DNA (smear positive sputum or culture growth)
- Time to results: ~1 week
- Target sequences: rpoB, katG, inhA, others
  - Xpert MTB-RIF (rpoB only)
  - Hain GenoType MDRTB (rpoB, inhA, katG)
  - WA DOH TB Lab (rpoB, katG, inhA, pncA)
  - CDC (all of the above, gyrA, emb, [injectables])
# Xpert MTB/RIF Test Performance for Diagnosis of Pulmonary TB

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear pos. TB</td>
<td>95-98%</td>
<td>99%</td>
</tr>
<tr>
<td>Smear neg. TB</td>
<td>60-72%</td>
<td></td>
</tr>
<tr>
<td>Rifampin “R”</td>
<td>98-99%</td>
<td>99-100%</td>
</tr>
</tbody>
</table>

Molecular Drug Susceptibility Testing

Typical Indications

- Treatment failure
- Previous treatment for active TB
- Known contact to confirmed case of MDR
- From a highly MDR-endemic setting (e.g., S. Africa, Baltic states, Russian prison)
### Drug resistance sequencing screen

**WA DOH PHL**

<table>
<thead>
<tr>
<th>DRSS</th>
<th>Result</th>
<th>Performed by</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH (inhA promoter)</td>
<td>Mutation NOT Detected</td>
<td>AXO</td>
<td>10/1/2015</td>
</tr>
<tr>
<td>INH (katG)</td>
<td>Mutation Detected</td>
<td>AXO</td>
<td>10/1/2015</td>
</tr>
<tr>
<td>301</td>
<td>Probably resistant to Isoniazid. [79.7% of resistant isolates in our evaluation of 173 clinical isolates have a mutation in the genetic regions evaluated with a positive predictive value of 96.7%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIF (rpoB)</td>
<td>Mutation Detected</td>
<td>AXO</td>
<td>10/1/2015</td>
</tr>
<tr>
<td>300</td>
<td>Probably resistant to Rifampin [97.5% of RIF-R isolates in our evaluation of 173 clinical isolates have a mutation in this gene with a positive predictive value of 95.1%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PZA (pncA)</td>
<td>Mutation NOT Detected</td>
<td>AXO</td>
<td>10/1/2015</td>
</tr>
<tr>
<td>305</td>
<td>Cannot rule out PZA resistance. [79.2% of resistant isolates in our in-house evaluation of 131 clinical isolates have a mutation in this gene with a positive predictive value of 90.5%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>306</td>
<td>DRSS results are preliminary, pending confirmation with culture-based DST methods. This specimen was tested with a sequencing-based research procedure that is not cleared or approved for diagnostic use by the U.S. Food and Drug Administration (FDA). Results obtained with DRSS are primarily for epidemiologic surveillance purposes and should not form the sole basis for treatment decisions. The assay will not detect mutations associated with resistance other than those located in the regions of M. tuberculosis genome screened AND that also have a well-established strong correlation with resistance. Thus, the absence of mutations does not necessarily indicate a lack of resistance since other mutations including those located in genomic regions not tested by the assay could confer resistance. Improper specimen collection/handling, the presence of inhibitors, and/or the presence of multiple mycobacterial strains in a specimen may also contribute to a false result.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


# Molecular detection of drug resistance

**CDC**

## Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 550 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td>Mutation: TCC&gt;TTG; Ser531Leu</td>
<td>Rifampin resistant. <em>(100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)</em></td>
</tr>
<tr>
<td>inhA (promoter)</td>
<td>No mutation</td>
<td>Isoniazid resistant. <em>(100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)</em></td>
</tr>
<tr>
<td>katG (ser315 codon)</td>
<td>Mutation: AGC-&gt;ACC; Ser315Thr</td>
<td>Ethambutol resistant. <em>(100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are EMB-R.)</em></td>
</tr>
<tr>
<td>embB (Met306_Gly408)</td>
<td>Mutation: ATG-&gt;GTG; Met306Val</td>
<td>Likely PZA resistant</td>
</tr>
<tr>
<td>pncA (promoter, coding region)</td>
<td>Mutation: 'A' inserted after nt192 Silent mutation: TCC-&gt;TCT; Ser65Ser</td>
<td>The Ser65Ser mutation detected is a synonymous (silent) single-nucleotide polymorphism (SNP) and does not result in an amino acid change and is not considered clinically significant.</td>
</tr>
<tr>
<td>gyrA (QRDR)</td>
<td>No mutation</td>
<td>Cannot rule out fluoroquinolone resistance. <em>(60% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)</em></td>
</tr>
<tr>
<td><em>rs</em> (1400 region)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). <em>(In our in-house evaluation of 550 clinical isolates:)</em></td>
</tr>
<tr>
<td><em>els</em> (promoter)</td>
<td>No mutation</td>
<td>* 91% of AMK-R isolates have a mutation in the rs locus;</td>
</tr>
<tr>
<td><em>tlyA</em> (entire ORF)</td>
<td>No mutation</td>
<td>* 87% of KAN-R isolates have a mutation in either the rs locus or the els locus;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* 55% of CAP-R isolates have a mutation in either the rs locus or the tlyA locus;</td>
</tr>
<tr>
<td>Drug (mcg/mL)</td>
<td>Result</td>
<td>Performed by</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>MGIT Pyrazinamide (PZA) 100</td>
<td>RESISTANT</td>
<td>CAL</td>
</tr>
<tr>
<td>MGIT Streptomycin (SM) 1.0</td>
<td>RESISTANT</td>
<td>CAL</td>
</tr>
<tr>
<td>MGIT Isoniazid (INH) 0.1</td>
<td>RESISTANT</td>
<td>CAL</td>
</tr>
<tr>
<td>MGIT Rifampin (RIF) 1.0</td>
<td>RESISTANT</td>
<td>CAL</td>
</tr>
<tr>
<td>MGIT Ethambutol (EMB) 5.0</td>
<td>Sensitive</td>
<td>CAL</td>
</tr>
</tbody>
</table>

119) Drug resistance to be confirmed by 7H-10 Plate Method. Performed at CDC
# DOH Plate DST Results

## Susceptibility Report - 2nd Line of Drugs

<table>
<thead>
<tr>
<th>Drug (mcg/mL)</th>
<th>Result</th>
<th>Percent Resistance</th>
<th>Colonies at 10^-3</th>
<th>Colonies at 10^-5</th>
<th>Performed by</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Control</td>
<td>Satisfactory</td>
<td>0</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Isoniazid 0.2</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Isoniazid 1.0</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Rifampin 1.0</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Streptomycin 2.0</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Streptomycin 10.0</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Ethionamide 5.0</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Ethambutol 5.0</td>
<td>Resistant</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Ethambutol 10.0</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>p-Aminosalicylic Acid 2.0</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Amikacin 6.0</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
<tr>
<td>Ofloxacin 1.0</td>
<td>Sensitive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CAL</td>
<td>6/23/2015</td>
</tr>
</tbody>
</table>
### CDC Plates DST Results

**Susceptibility Testing Method:** Indirect agar proportion, 7H10 medium; Susceptibility is defined as < 1% resistance compared to colonies that develop on drug-free media

### RESULTS:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percent Resistance</th>
<th>Interpretation</th>
<th>Percent Resistance</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid 0.2 µg/ml</td>
<td>100</td>
<td>R</td>
<td>Kanamycin 5.0 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Isoniazid 1.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td>Ethionamide 10.0 µg/ml</td>
<td>50</td>
</tr>
<tr>
<td>Isoniazid 5.0 µg/ml</td>
<td>12.50</td>
<td>R</td>
<td>Capreomycin 10.0 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Rifampin 1.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td>PAS 2.0 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Ethambutol 5.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td>Ofloxacin 2.0 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin 2.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td>Amikacin 4.0 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin 10.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifabutin 2.0 µg/ml</td>
<td>100</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin 2.0 µg/ml</td>
<td>0</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide 100 µg/ml</td>
<td></td>
<td></td>
<td>Resistant</td>
<td></td>
</tr>
</tbody>
</table>

**Susceptibility Testing Method:** MGIT 960
TB Diagnosis Summary--1

- Cough, sputum, fever, night sweats, weight loss sensitive but not specific
- Initial diagnostic test is imaging
- Collect appropriate specimens when clinical and imaging findings suggest TB
- Mycobacteriology: AFB smear, culture, and nucleic acid amplification
- Other tests: cytology/pathology, cell count, differential, protein, LHD, glucose, pH
TB Diagnosis Summary--2

- **Cautious use:**
  - TST, IGRA
  - ADA in serosal fluids
- **Medical decision making ingredients:** clinical, radiography, laboratory results AND epidemiology
- **Predictive value of a test** is driven by both test accuracy AND pre-test probability
- **Specialty evaluation** indicated for patients with inconclusive findings
Cases
Case 1 Clinical Presentation

• Cough, chest pain, fever, anorexia x 6 weeks
• What is your next step?
Case 1: What Would You Do?

A. Place a TST
B. Collect sputum--AFB smear/culture/PCR
C. CT chest
D. PA/LAT CXR
E. Levofloxacin 500mg po qd x 7d
Case 1: Chest Radiographs
2007 vs 2013
Case 1—Evaluation

- Sputum AFB smears 1+ on 2 separate specimens
- What else?
Case 1: NAAT

• Positive for MTB
• Negative for MAC
Case 2—Clinical Presentation

• 33 y/o Ethiopian male
• Visited home recently for 3 months
• Malaise, fatigue, fever x 1 month
• Left chest pain worsening over past 2-3 weeks
• Weight 63kg → 60kg
• T 38.5°C; left base dull to percussion with decreased breath sounds
Case 2—Chest Radiograph
Case 2—Initial Evaluation

- HIV negative
- Hgb 10, MCV 80, albumin 3.1
- TST placed
Case 2—What Would You Do?

What would you do next?

A. Complete sputum collection and start 4-drug therapy

B. Thoracentesis, sputum collection, and pleural biopsy if other specimens AFB smear-negative

C. Give azithromycin for presumed CAP and discharge to outpatient pulmonary follow-up

D. Refer to thoracic surgery for tube drainage
Case 2—Pleural Fluid

- Protein 4.4 gm/dL,
- WBC ~1500 (65% lymphocytes)
- No AFB seen
- Pleural biopsy: necrotizing granulomata without visible AFB (culture pending, PCR not done)
- TST 18mm
Questions? Comments?

christopher.spitters@kingcounty.gov