DIAGNOSING ACTIVE TB & DRUG RESISTANCE

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McGill University

Case 1 – Mr. M

- 28 yr male, originally from China
- 10 days progressive cough, productive
- Fever, malaise, anorexia

- Otherwise healthy
- Immigrated to Canada 2 years ago
- Father had history of TB

TB?
Case 2 – Mr. N

- 60 yr male, Canadian aboriginal
- 3 months chronic cough, chest pain
- Weight loss ~15lbs, night sweats
- Type II DM, chronic renal failure, smoker
- History of incarceration
- Does not recall contact with any TB cases

TB?

Case 3 – Ms. O

- 32 yr female, second generation Canadian (parents from Ghana)
- Gradual onset swelling on L side neck
- Fatigue, myalgias, arthralgias
- Treated for STDs 1 yr ago – chlamydia, syphilis
- Grandmother died of TB, minimal contact

TB?
Who has TB?

- Mr. M (28 yr M) → Influenza
- Mr. N (60 yr M) → Lung Ca
- Ms. O (31 yr F) → SLE

“Typical” clinical picture of active TB

- Epidemiologic Risk Group
  - Foreign born, aboriginal Canadians, elderly, close contacts
  - HIV, immunosuppression (TNF-alpha), malnutrition, end-stage renal disease, diabetes
  - IVDU, substance abuse, incarceration, homelessness, healthcare workers, smoking

- Symptoms
  - Chronic cough >3 weeks (dry → productive), fevers, night sweats, hemoptysis, anorexia, weight loss, chest pain
  - Extrapulmonary disease mimics site specific differential diagnoses

- Signs
  - Most commonly = completely normal examination
  - Bronchial breathing, rales in advanced disease
  - Lymphadenopathy, pleural effusions, bone/joint involvement

- Radiology
Microbiologic Confirmation

- Smear microscopy
- Pathology
- Culture
- NAAT
- Drug Susceptibility Testing

Specimens

- Sputum
  - Labelled, leak-proof container
  - Collect in well-ventilated area
  - Carefully explain process to patient
  - Rinse mouth with water
  - Refrigerate if possible
  - Rapid transport to lab
Specimens

- Induced sputum
  - Patient inhales solution of hypertonic saline
  - Induces coughing, loosens (and dilutes) secretions

- Bronchial wash/
  Bronchoalveolar lavage
  - Invasive endoscopic procedure
  - Read: scope down trachea into lungs

Specimens

- Endoscopic gastric lavage
  - Invasive endoscopic procedure
  - Read: scope down esophagus into stomach

- Gastric String Test
  - Less invasive, still uncomfortable
  - Requires less expertise, no equipment
  - Read: patient swallows pill attached to string, wait ~2hrs, pull string out and culture intragastric portion
Smear Microscopy

- 60-70% sensitive, very specific
- Quick, cheap, relatively easy
- Stains take advantage of mycolic acid in cell walls of Mycobacteria
- “Acid Fast Bacilli”
- Stain → Decolorize → Counterstain

<table>
<thead>
<tr>
<th>LIGHT MICROSCOPY</th>
<th>FLUORESCENT MICROSCOPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corbol fuschin (+ heat = Ziehl-Neelsen) (- heat = Kinyoun)</td>
<td>Phenolic Auramine or Auramine - Rhodamine</td>
</tr>
<tr>
<td>Acid alcohol</td>
<td>Acid alcohol</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>Potassium permanganate</td>
</tr>
</tbody>
</table>

Stain → Decolorize → Counterstain
<table>
<thead>
<tr>
<th>LIGHT MICROSCOPY</th>
<th>FLUORESCENT MICROSCOPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Traditional method</td>
<td>□ Need lower magnification $\rightarrow$ 45% less time to examine slides</td>
</tr>
<tr>
<td>□ More experienced microscopists</td>
<td>□ 10% more sensitive</td>
</tr>
<tr>
<td>□ More specific?</td>
<td>□ Easier staining procedure?</td>
</tr>
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</table>

Policies on Smear Microscopy

**Definition of a new sputum smear-positive TB case**

The revised definition of a new sputum smear-positive pulmonary TB case is based on the presence of at least one acid-fast bacilli (AFB+) in at least one sputum sample in countries with a well-functioning external quality assurance (EQA) system.

2007

**Reduction of number of smears for the diagnosis of pulmonary TB**

WHO recommends the number of specimens to be examined for screening of TB cases can be reduced from three to two, in places where a well-functioning external quality assurance (EQA) system exists, where the workload is very high and human resources are limited.

2007

Front Loaded Specimen Collection?  2009
Sputum Processing?  
LED Fluorescent Microscopy?

Fluorescent LED Microscopy

- Higher Sensitivity than ZN (and possibly conventional FM)
- 46% time savings vs. ZN (equivalent to conventional FM)
- Advantages of FM but less expensive, requires less maintenance, no need for a dark room

Minion et al. unpublished

Commercial LED Microscopes

<table>
<thead>
<tr>
<th>Device</th>
<th>Manufacturer</th>
<th>Translation (Microscope)</th>
<th>Attachment</th>
<th>Light Emission (Type)</th>
<th>Battery Powered</th>
<th>Weight (g)</th>
<th>Cost (US$)</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Prime Star</td>
<td>Cell2mis, Griesheim, Germany</td>
<td>Yes, NA</td>
<td>EpiFluorescent</td>
<td>Yes</td>
<td>5.95</td>
<td>4.25</td>
<td>(Ref)</td>
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<tr>
<td>Luxel®</td>
<td>UK Scientific, Berkshire, PA, USA</td>
<td>No</td>
<td>Objective lens (18), 40, 60 and 100x oil</td>
<td>EpiFluorescent</td>
<td>6.466</td>
<td>106-2066</td>
<td>(Ref)</td>
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<tr>
<td>ProView</td>
<td>OBC Diagnostics, PA, USA</td>
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<td>Objective lens (40, 60 and 100x oil)</td>
<td>EpiFluorescent</td>
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<td>9999</td>
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<tr>
<td>FluorLED</td>
<td>Perst Corporation, Solna, Sweden</td>
<td>No</td>
<td>Adapter attached to base and other installation head of microscope</td>
<td>Transfluorescent</td>
<td>5</td>
<td>1897-2853</td>
<td>(Ref)</td>
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<tr>
<td>CyScope®</td>
<td>Perkin-Elmer, USA</td>
<td>Yes, NA</td>
<td>EpiFluorescent</td>
<td>Yes</td>
<td>2.7</td>
<td>2370-3889</td>
<td>(Ref)</td>
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</tbody>
</table>

Other Potential Innovations

Pathology

- Caseating Granulomas
- Acid fast stain confirmation
- Provides important evidence in extrapulmonary disease
- TB not suspected, but tissue sent to pathology
**Culture**

- Considered gold standard for diagnosis
- High sensitivity
- Slow turnaround time, relatively expensive
- Requires specimen processing
- Biosafety

**Decontamination**

- *M. tuberculosis* is slow growing
- Without decontamination, normal flora would overgrow cultures
- *M. tuberculosis* is relatively hard to kill
- Aim for ~5% cultures contaminated

Liquefication – N-acetyl-L-cysteine

Decontamination – 1-5 % NaOH

Neutralization – Phosphate buffer

Centrifugation

Culture of Sediment (processed smear)
### SOLID MEDIA

- **Egg Based:**
  - Lowenstein-Jensen
  - Ogawa
  - Petragnani
  - ATS medium

- **Non-egg Based:**
  - BD, Middlebrook 7H-10
  - BD, Middlebrook 7H-11 (+casein)

  - Slow turnaround time (4 – 8 weeks)
  - Colony morphology helpful for speciation

### LIQUID MEDIA

- **Manual Detection:**
  - BD, MGIT
  - Septi-Check AFB

- **Automated Detection:**
  - BD, MGIT 960
  - BD, BACTEC 460
  - Biomerieux, MB/BacT
  - TREK, ESP Culture System II

  - Detection of growth (e.g. MGIT): oxygen quenches fluorescent compound → organisms deplete oxygen and fluorescence is detected

  - 10% higher sensitivity (also higher contamination)

  - Faster turnaround time (1 – 4 weeks)

  - Greater biosafety risk

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**Policy on Culture-based Diagnostics**

The use of liquid medium for culture and DST

WHO recommends, as a step-wise approach:

1. The use of liquid medium for culture and DST in middle- and low-income countries.
2. The rapid species identification to address the needs for culture and drug susceptibility testing (DST).

Taking into consideration that liquid systems will be implemented in a phased manner, integrated into a country specific comprehensive plan for laboratory capacity strengthening and addressing the following two issues:

1. Appropriate biosafety level;
2. Detailed customer plan describing guarantees and commitments of the manufacturer;
3. Training of staff;
4. Maintenance of infrastructure and equipment in laboratories;
5. Quick transportation of samples from the peripheral to the culture laboratory;
6. Rapid communication of results.

2007
NAAT

- High specificity and PPV

- Sensitivity is lower and highly variable
  - Especially in extrapulmonary specimens
  - Especially in smear negative specimens
    (>95% smear + vs. 40-80% smear -)

- Expensive

- Should not be used on follow-up specimens

- In house assays
  - Roche Cobas AmpliCor
  - Gen-Probe AMTD
  - BD ProbeTec ET
2009 Updated CDC Guidelines

Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

Updated Recommendation
NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities. The following testing and interpretation algorithm is proposed.

Culture Negative TB

- As much as 20% of active TB diagnoses fail to have microbiologic confirmation
- Pediatric TB, extrapulmonary TB
- Reliant on clinical suspicion, radiology, pathology, TST/IGRA...?
- Monitor closely response to treatment
Detecting Drug Resistance

- Foreign born, countries with high prevalence of resistance
- Contact of drug-R case
- Relapsed cases, previously treated with anti-TB drugs
- Treatment failure, treatment default

Types of Drug Resistance

- **Mono-resistant**: Resistance to a single drug
- **Poly-resistant**: Resistance to more than one drug, but not the combination of isoniazid and rifampicin
- **Multidrug-resistant (MDR)**: Resistance to at least isoniazid and rifampicin
- **Extensively drug-resistant (XDR)**: MDR plus resistance to fluoroquinolones and at least 1 of the 3 injectable drugs (amikacin, kanamycin, capreomycin)
Types of Drug Resistance

- **Primary drug-resistance: “New Cases”**
  Drug resistance in a patient who has never been treated for tuberculosis or received less than one month of therapy

- **Secondary (acquired) drug-resistance: “Previously Treated Cases”**
  Drug resistance in a patient who has received at least one month of anti-TB therapy

Drug Susceptibility Testing

- **MTB grows in heterogeneous populations**

- **Expect 1:10^5-10^8 bacteria to be resistant**

- **Patient with pulmonary cavitation has 10^7-10^9 bacillary load**

- **>1% resistant bacteria results in clinically relevant resistance**
Drug Susceptibility Testing

- **Agar Proportion Method**
  - Plate isolate onto drug-free and drug-containing media
  - Count colonies on each – if >1% of drug-free growth present on drug-containing growth = Resistant

- **BACTEC 460/MGIT**
  - Inoculate drug-containing bottles
  - Inoculate drug-free bottle with 1:100 diluted isolate
  - Growth Index (growth units) compared

Line Probe Assays

- Detection of MTB & RIF-resistance (rpoB)
  - Requires extraction, amplification
  - Colorimetric development using immobilized probes

- Innogenetics, INNO-LiPA Rif TB
- Hain, GenoType MTBDRplus
Policy on Line Probe Assays

WHO policy statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis

Policy on Novel Culture-based Diagnostics?

- MODS?
- TLA?
- NRA?
- CRI?
- Phage?
Microscopically Observed Drug Susceptibility Testing (MODS)

- Direct inoculation of patient specimens – detection & DST
- Liquid culture – improved sensitivity
- Microcolony detection – faster turnaround time
- Biosafety?
- Specificity of ID?

Thin Layer Agar (TLA)

- Direct inoculation of patient specimens – detection & DST
- Solid media – easier to manipulate
- Microcolony detection – faster turnaround time
- Biosafety?
- Specificity of ID?
### Nitrate Reductase Assay (NRA)

- aka Griess method
- Based on MTB’s ability to reduce nitrate to nitrite
- Simple
- Sensitive detection of small amount of metabolic byproduct improves turnaround time
- Prevalence of nitrate reductase negative strains of MTB?

1. **KNO$_3$ - containing media**
2. Add reagent to drug-free slant day 7 (repeat day 10, 14)
3. Color development = growth

### Colorimetric Redox Indicators (CRI)

- Based on reduction of indicator by actively growing MTB
- MIC determination using microdilution
- Detection of active metabolism improves turnaround time
- Biosafety concerns?
- Suitable for reference labs?

1. Incubate microdilution plate 7 days
2. Add indicator to all wells, incubate overnight
3. Color change = growth
Mycobacteriophage Assays (FASTPlaque™)

- Based on amplification of phage viruses in live MTB
- 2 day turnaround time for detection & DST, minimal biosafety concerns
- BUT...
- High rates of contaminated or uninterpretable tests
- High rates of false positives

MDR-XDRTB Color Test for Regional Laboratories*

1. Liquefaction & decontamination in transport medium at room temperature
2. Direct application of 2 drops to selective thin layer agar for incubation in room air for MDRIB testing & XDRTB screening
3. Color growth detection & microscopy confirmation of morphology

Biosafety similar to sputum microscopy because sputum is smeared directly onto the plate which is then permanently double-sealed until autoclaving

*Carlton Evans, Welcome Trust, Peru
Loop Mediated Isothermal Amplification (LAMP)

- Simplified NAAT, does not require a thermocycler, detection by fluorescence
- Sensitivity 97%
  Specificity 99%
  (culture reference)
- Rapid (1 hour), high throughput
- Feasible in high burden settings?

Eiken Chemical Co., Tokyo, Japan

GeneXpert® MTB/RIF Test

Workflow
- sputum
- simple 1-step external sample prep. procedure
- time-to-result < 2 h
- throughput ≥ 16 tests / day / module
- no need for biosafety cabinet
- integrated controls
- true random access

Performance
- specific for MTB
- sensitivity better than smear, similar to culture
- detection of rif-resistance via rpoB gene

Product and system design
- test cartridges for GeneXpert System
- several GeneXpert modules can be combined in 1 workstation
- swap replacement of detection unit
- ∼1 day technician training for non-mycobacteriologists
Serology

- Attractive ... Especially if point of care (POC) option
- >80 antigenic targets evaluated and several commercial assays developed
- All existing serologic tests have failed to demonstrate adequate accuracy
  - Although still marketed and sold by many companies and used in developing countries!

A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis

Thorax 2007

Commercial Serological Antibody Detection Tests for the Diagnosis of Pulmonary Tuberculosis: A Systematic Review

PloS Medicine 2007

Performance of Purified Antigens for Serodiagnosis of Pulmonary Tuberculosis: a Meta-Analysis

Clin Vaccine Immunol 2009

WHO/TDR evaluation of 19 commercial serologic tests for TB: poor accuracy

Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis (all patients, n=355)


WHO/TDR Diagnostics Evaluation Series 2009
**Antigen Detection**

- **Urinary Lipoarabinomannan (LAM)**
  - ELISA-based test
  - ClearviewTB (Inverness, UK)
  - Optimal specimen, rapid turnaround (2.5 hrs)
  - Potential for POC “dipstick”

- Initially evaluations were promising
  - Boehme et al. 2005: 80% sensitivity; 99% specificity

- BUT ...

- Subsequent studies have failed to demonstrate similar performance

- Indicated for HIV+?
  - Improved sensitivity with low CD4?

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**Disappointing LAM performance**

![Diagram showing performance metrics for LAM antigen detection](image_url)

- **Sensitivity (95% CI)**
  - Hamzei et al. (2001): 0.90 (0.85 - 0.95)
  - Tesserreta et al. (2002): 0.97 (0.95 - 0.99)
  - Gillies et al. (2001): 0.90 (0.85 - 0.95)
  - Shin et al. (2002): 0.85 (0.79 - 0.92)
  - Varrin et al. (2005): 0.95 (0.92 - 0.98)
  - Lin et al. (2005): 0.95 (0.92 - 0.98)
  - Dalrymple et al. (2006): 0.93 (0.88 - 0.97)
  - Ghedia et al. (2008): 0.93 (0.88 - 0.97)

- **Positively Specificity (95% CI)**
  - Hamzei et al. (2001): 0.53 (0.31 - 0.75)
  - Tesserreta et al. (2002): 0.69 (0.64 - 0.74)
  - Gillies et al. (2001): 0.70 (0.65 - 0.75)
  - Shin et al. (2002): 0.71 (0.66 - 0.76)
  - Varrin et al. (2005): 0.94 (0.92 - 0.96)
  - Lin et al. (2005): 0.94 (0.92 - 0.96)
  - Dalrymple et al. (2006): 0.94 (0.92 - 0.96)
  - Ghedia et al. (2008): 0.94 (0.92 - 0.96)

- Minjon et al. unpublished
Global TB Case Detection

- 2.6 million new smear + cases notified in 2007
- 64% of the estimated 4.1 million cases
- 5.3 million new cases overall notified in 2007
- 57% of the estimated 9.3 million cases

WHO Report 2009 – Global Tuberculosis Control

Conclusions

- In Canada ...
  - You will not diagnose TB if you are not looking for it
  - Include on differential diagnosis of any patient with epidemiologic risk factors and compatible clinical syndrome
  - Use laboratory wisely

- Globally ...
  - Need better access to diagnostics
  - Urgent need for simple, cheap, accurate tests for detection and DST