DIAGNOSING ACTIVE TB & DRUG RESISTANCE

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Epidemiology MSc program
McGill University

Montreal TB Course
Oct 30th 2009

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

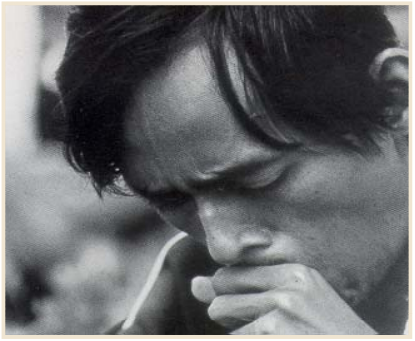
- Epidemiologic Risk Group
  - Foreign born, aboriginal Canadians, elderly, close contacts
  - HIV, immunosuppression (TNF-alpha), malnutrition, end-stage renal disease, diabetes
  - IVDU, substance abuse, incarceration, homelessness, healthcare workers, smoking
- Symptoms
  - Chronic cough >3 weeks (dry → productive), fevers, nightsweats, 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

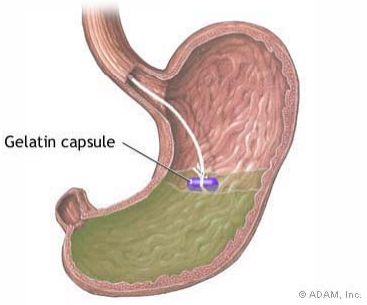
- Induced sputum
  - Patient inhales solution of hypertonic saline
  - Induces coughing, loosens (and dilutes) secretions

- 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

LIGHT MICROSCOPY

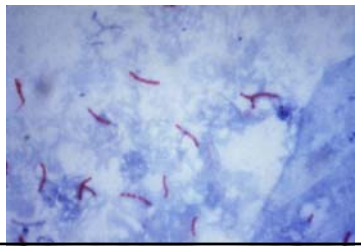
Carbol fuschin
(+ heat = Ziehl-Neelsen)
(- heat = Kinyoun)

↓

Acid alcohol

↓

Methylene blue



STAIN

↓

DECOLORIZE

↓

COUNTERSTAIN

FLUORESCENT MICROSCOPY

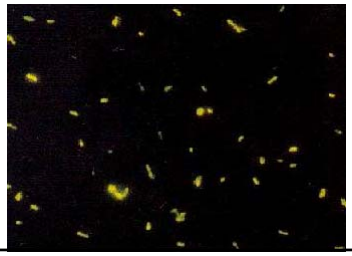
Phenolic Auramine or
Auramine -Rhodamine

↓

Acid alcohol

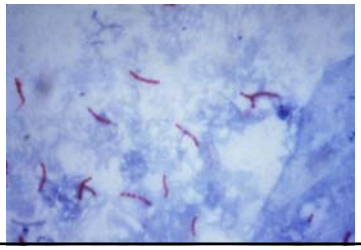
↓

Potassium
permanganate



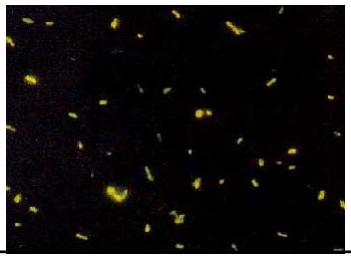
LIGHT MICROSCOPY

- Traditional method
- More experienced microscopists
- More specific?



FLUORESCENT MICROSCOPY

- Need lower magnification → 45% less time to examine slides
- 10% more sensitive
- Easier staining procedure?



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

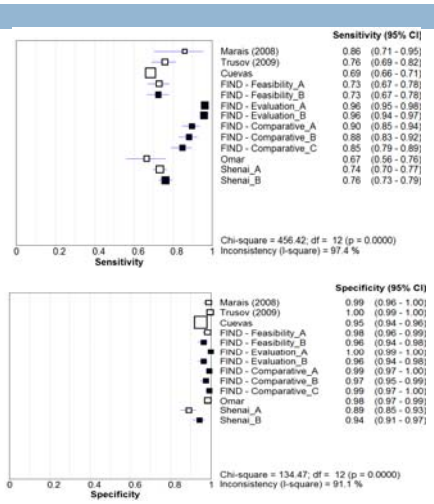
Front Loaded Specimen Collection?
Sputum Processing?
LED Fluorescent Microscopy?

2009

http://www.who.int/tb/dots/laboratory/policy/en/

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 1. Comparison of commercial light-emitting diode products currently available for TB diagnostics.

Device	Manufacturer	Standalone microscope	Attachment	Light transmission	Battery powered	Weight (kg)	Cost (US $)	Ref.
Primo Star iLED	Carl Zeiss, Oberkochen, Germany	Yes	NA	Epifluorescent	Yes	9.5	4825*	[101]
Lumin™	LW Scientific, Lawrenceville, GA, USA	No	Objective lens replacement (20, 40, 60 and 100× oil)	Epifluorescent	Yes	0.448	700–2000*	[102]
ParaLens	QBC™ Diagnostics, Philipsburg, PA, USA	No	Objective lens replacement (40, 60 and 100× oil)	Epifluorescent	Yes	1.27	995‡	[103]
FluoLED	Fraen Corporation Srl, Settimo Milanese, Italy	No	Adaptor attached to base and filter installed on head of microscope	Transfluorescent	Yes	5	1977–3530¶	[104]
CyScope®	Partec, Gorlitz, Germany,	Yes	NA	Epifluorescent	Yes	2.7	2372–3699#	[105]

Minion et al. *Exp Rev Med Dev* 2009

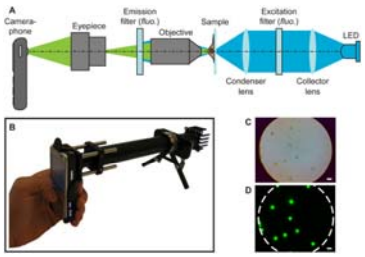
Other Potential Innovations

OPEN ACCESS Freely available online PLoS one

Mobile Phone Based Clinical Microscopy for Global Health Applications

David N. Breslauer[1,2*], Robi N. Maamari[2*], Neil A. Switz[3], Wilbur A. Lam[2,4], Daniel A. Fletcher[1,2,3*]

1 UCSF/UC Berkeley Bioengineering Graduate Group, 2 Department of Bioengineering, University of California Berkeley, Berkeley, California, United States of America, 3 Biophysics Graduate Group, University of California Berkeley, Berkeley California, United States of America, 4 Department of Pediatrics, University of California San Francisco, San Francisco, California, United States of America



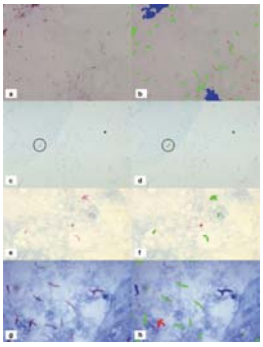


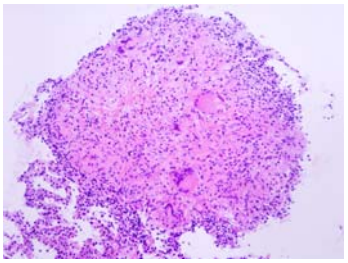
Image processing techniques for identifying *Mycobacterium tuberculosis* in Ziehl-Neelsen stains

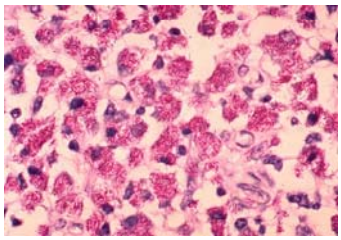
P. Sadaphal,*† J. Rao,‡ G. W. Comstock,*† M. F. Beg‡

* Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, † University Research Co., LLC, Bethesda, Maryland, USA; ‡ School of Engineering Science, Simon Fraser University, Burnaby, British Columbia, Canada

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

↓

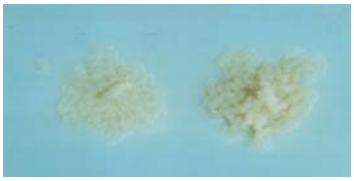
Centrifugation

↓

Culture of Sediment
(processed smear)

SOLID MEDIA

- Egg Based:
  - Lowenstein-Jensen
  - Ogawa
  - Petragnani
  - ATS medium
- Non-egg Based:
  - BD, Middlebrook 7H-10
  - BD, Middle brook 7H-11 (+casein)
- Slow turnaround time (4 – 8 weeks)
- Colony morphology helpful for speciation



LIQUID MEDIA

- Manual Detection:
  - BD, MGIT
  - Septi-Chek 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

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).

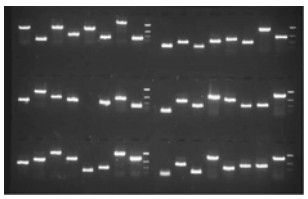
Taking into consideration that liquid systems will be implemented in a phased manner, integrated into a country specific comprehensive plan for laboratory capacity strengthening and addressing the following key issues:

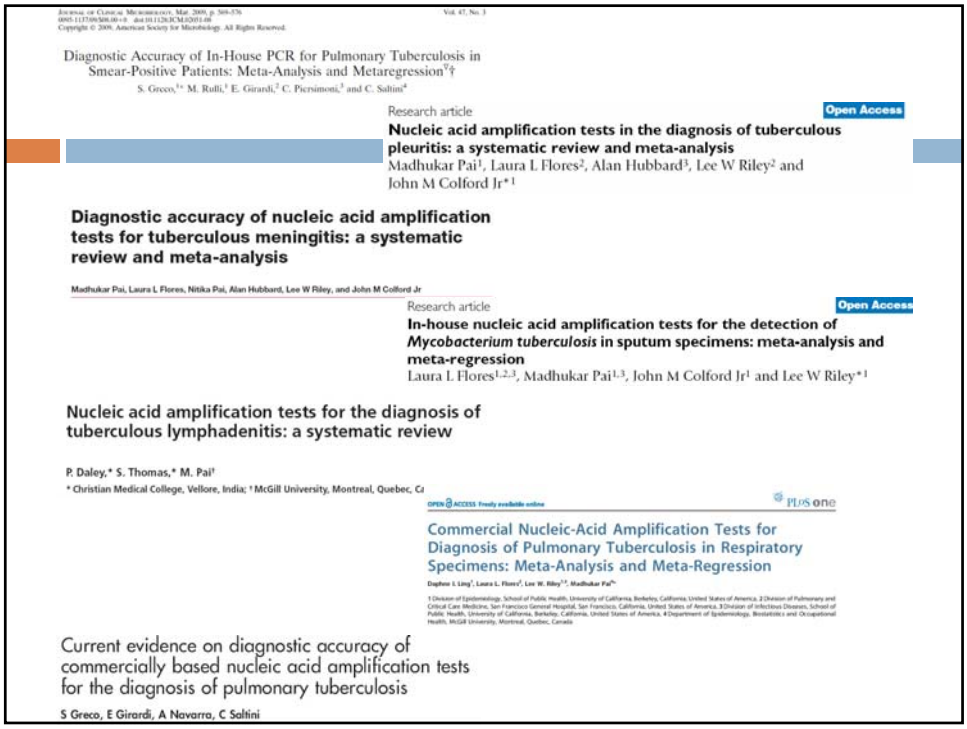
1. **Appropriate biosafety level;**
2. **detailed customer plan describing guarantees and commitments of the manufacturer;**
3. **appropriate training of staff;**
4. **maintenance of infrastructure and equipment in laboratories;**
5. **quick transportation of samples from the peripheral to the culture 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





Diagnostic Accuracy of In-House PCR for Pulmonary Tuberculosis in Smear-Positive Patients: Meta-Analysis and Metaregression

Research article Open Access

Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis

Madhukar Pai[1], Laura L Flores[2], Alan Hubbard[3], Lee W Riley[2] and John M Colford Jr*[1]

Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis

Madhukar Pai, Laura L Flores, Nitika Pai, Alan Hubbard, Lee W Riley, and John M Colford Jr

Research article Open Access

In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression

Laura L Flores[1,2,3], Madhukar Pai[1,3], John M Colford Jr[1] and Lee W Riley*[1]

Nucleic acid amplification tests for the diagnosis of tuberculous lymphadenitis: a systematic review

P. Daley,* S. Thomas,* M. Pai†

* Christian Medical College, Vellore, India; † McGill University, Montreal, Quebec, Ca

PLoS one

Commercial Nucleic-Acid Amplification Tests for Diagnosis of Pulmonary Tuberculosis in Respiratory Specimens: Meta-Analysis and Meta-Regression

Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis

S Greco, E Girardi, A Navarra, C Saltini

2009 Updated CDC Guidelines

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

Updated Recommendation

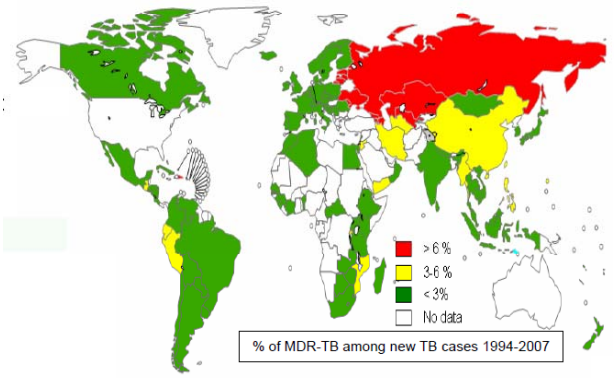
NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities. The following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

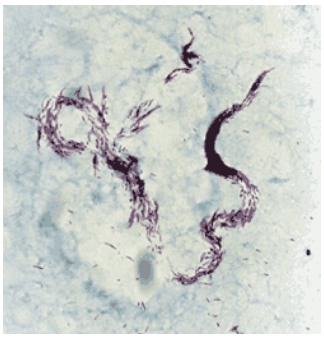
  Drug resistance in a patient who has never been treated for tuberculosis or received less than one month of therapy

- **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



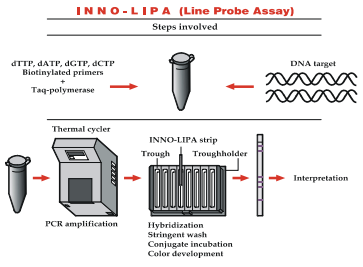
Drug Susceptibility Testing

- 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

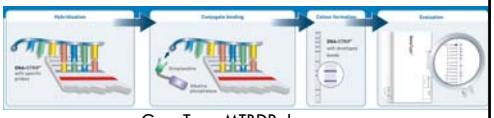
- BACTEC 460/MGIT
  - Inoculate drug-containing bottles
  - Inoculate drug-free bottle with 1:100 diluted isolate
  - Growth Index (growth units) compared

Line Probe Assays

- Detection of MTB & RIF-resistance (*rpo*B)
- Requires extraction, amplification
- Colorimetric development using immobilized probes
- Innogenetics, INNO-LiPA Rif TB
- Hain, GenoType MTBDRplus



Inno-LiPA Rif.TB assay
Innogenetics, Belgium



GenoType MTBDRplus assay
Hain Lifescience GmbH, Germany

Policy on Line Probe Assays

World Health Organization

2008

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

GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis

D.I. Ling*, A.A. Zwerling* and M. Pai*,#

Expert Reviews

Rapid diagnosis of drug-resistant TB using line probe assays: from evidence to policy

Daphne I Ling, Alice A Zwerling and Madhukar Pai

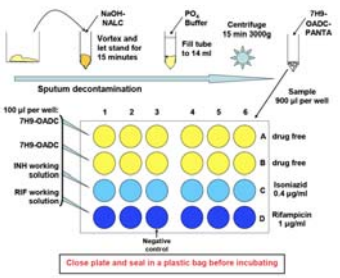
Policy on Novel Culture-based Diagnostics?

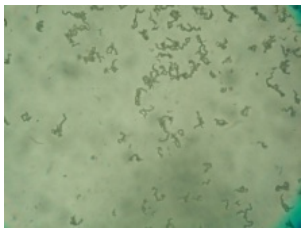
- MODS?
- TLA?
- NRA?
- CRI?
- Phage?

2009

Microscopically Observed Drug Susceptibility Testing (MODS)

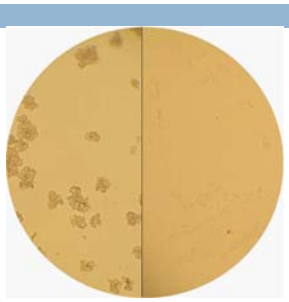
- Direct inoculation of patient specimens – detection & DST
- Liquid culture – improved sensitivity
- Microcolony detection – faster turnaround time
- Biosafety?
- Specificity of ID?

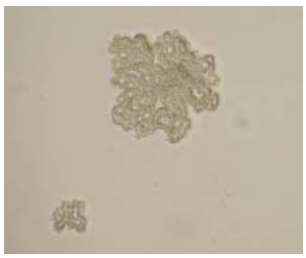




Thin Layer Agar (TLA)

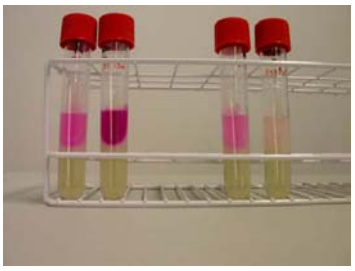
- Direct inoculation of patient specimens – detection & DST
- Solid media – easier to manipulate
- Microcolony detection – faster turnaround time
- Biosafety?
- Specificity of ID?





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 biproduct improves turnaround time
- Prevalence of nitrate reductase negative strains of MTB?



KNO_3 - containing media

↓

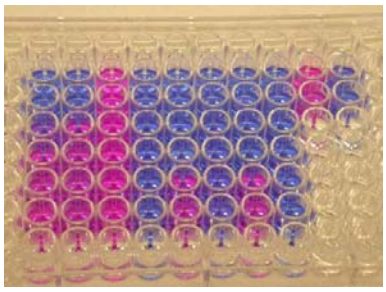
Add reagent to drug-free slant day 7 (repeat day 10, 14)

↓

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?



Incubate microdilution plate 7 days

↓

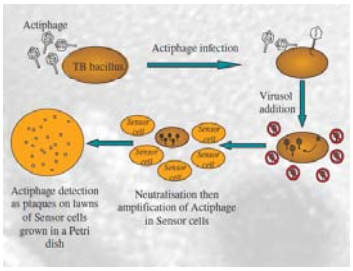
Add indicator to all wells, incubate overnight

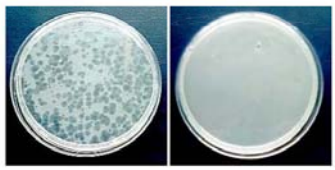
↓

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





Plaques – viable MTB cells present

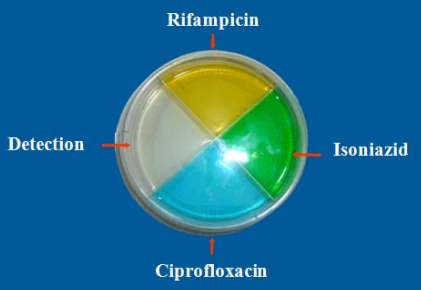
No plaques – no viable MTB cells present

MDR-XDRTB Color Test for Regional Laboratories*

FIND foundation for innovative new diagnostics

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 MDRTB testing & XDRTB screening



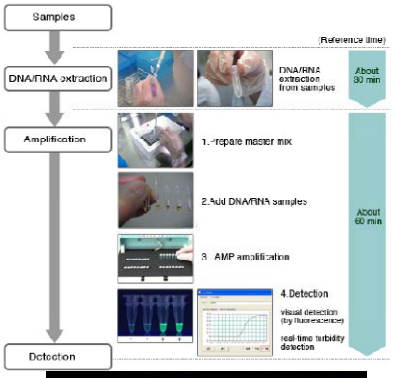
3 Color growth detection & microscopy confirmation of morphology

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

GeneXpert System module

Cepheid

cartridge

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

Karen R Steingart, Megan Henry, Suman Laal, Philip C Hopewell, Andrew Ramsay, Dick Menzies, Jane Cunningham, Karin Weldingh, Madhukar Pai

Thorax 2007

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

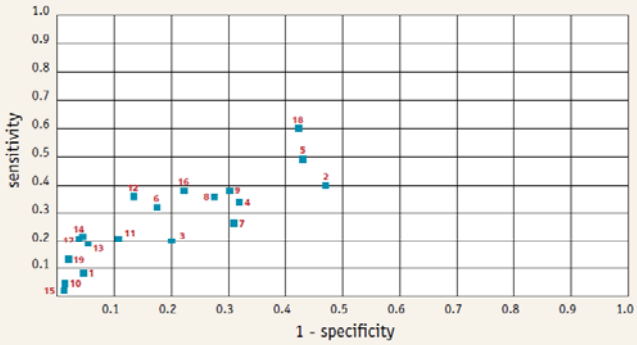
PLoS Medicine 2007

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)



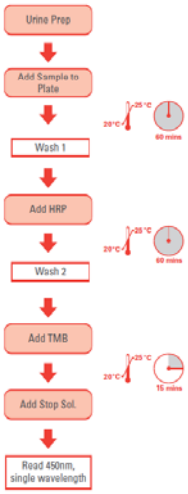
1. ABP Diagnostics 2. Advanced Diagnostics 3. American Bionostica 4. Ameritek USA 5. Bio-Medical 6. Chembio Diagnostic Systems 7. CTK Biotech 8. Hema Diagnostic Systems 9. Laboratorios Silanes

WHO/TDR Diagnostics Evaluation Series 2009

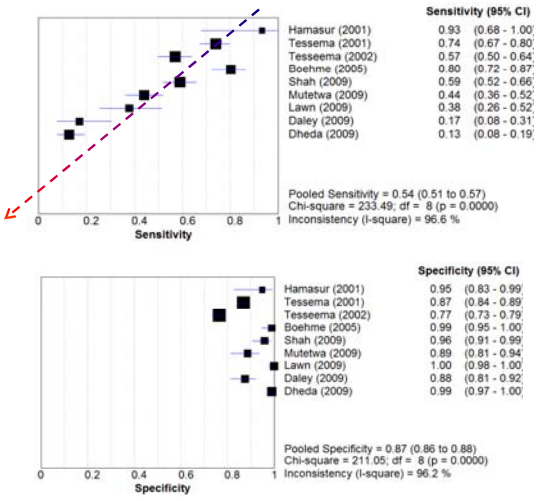
Antigen Detection

Clearview®

- **Urinary Lipoarabinomannan (LAM)**
  - ELISA-based test
  - Clearview(R) TB (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?

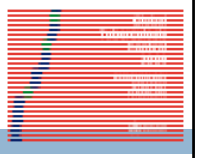


Disappointing LAM performance

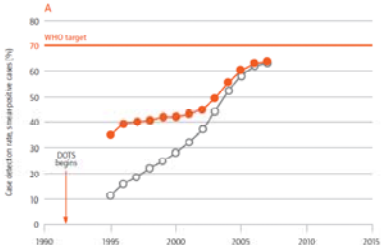


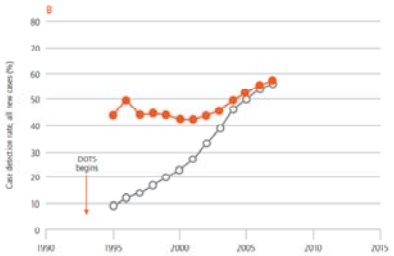
Minion et al. unpublished

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

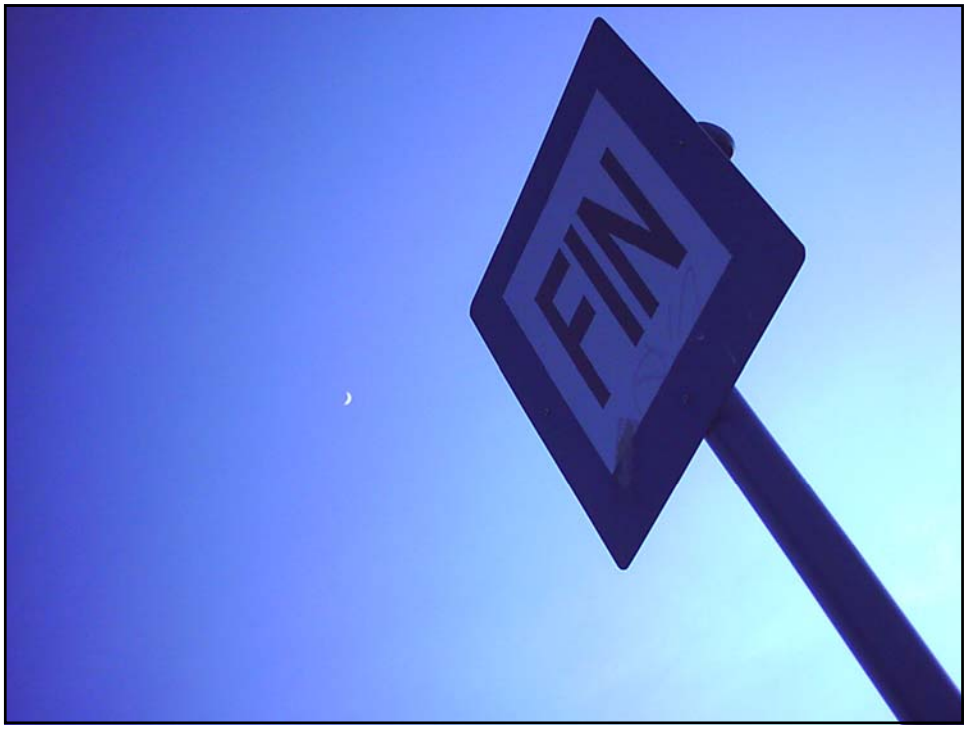




WHO Report 2009 – Global Tuberculosis Control

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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