Recent Progress in Diagnosis of Tuberculosis

結核病診斷之新進展

Traditional Methods for Diagnosis of Tuberculosis

- **Presumptive**

  Clinical, radiological, AFB microscopy, tuberculin test, pathological

- **Definitive**

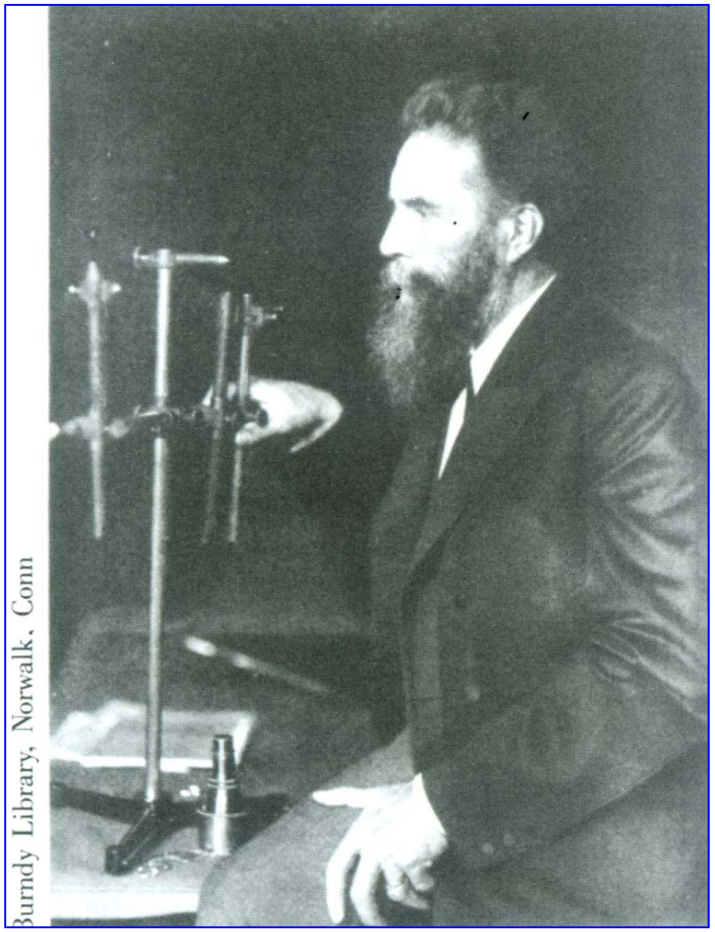
  Isolation and identification of *Mycobacterium tuberculosis*

Progress in TB Diagnosis?

Past	Present
Koch discovered tubercle bacillus 127 years	No major discovery Except TB Genome, IS*6110*, BACTC 460 (Liquid media)
TB diagnosed by symptoms - pre-historic	Still the same practice in many high burden countries
Tuberculin test - >100 years	Still commonly used
Egg-based media - ~ 100 years	Still most commonly used
AFB smear for diagnosis – 127 years	Still the major diagnostic tool in many countries
Radiologic diagnosis	Still the important tool, CT

Progress in Radiologic Diagnosis of Tuberculosis 1

- In 1895 Roentgen WC discovered X-Ray
- In 1901, he became the first recipient of the Novel Prize for Physics



Photograph taken in 1906

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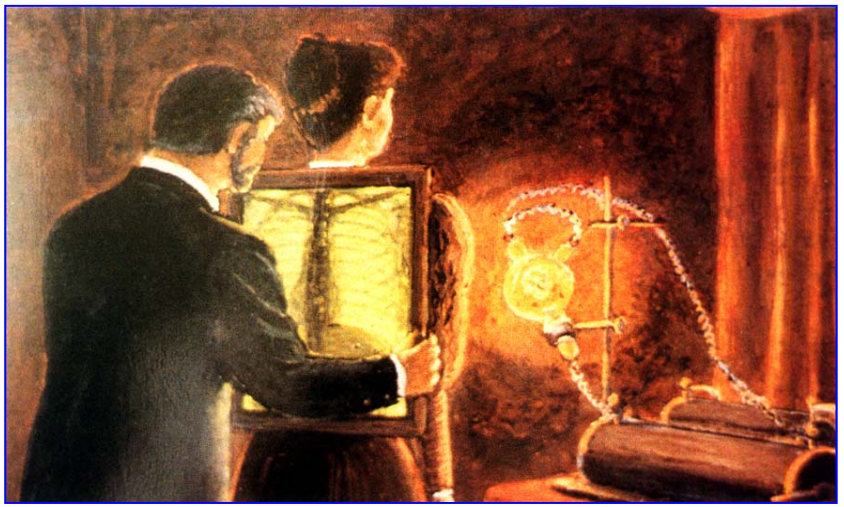
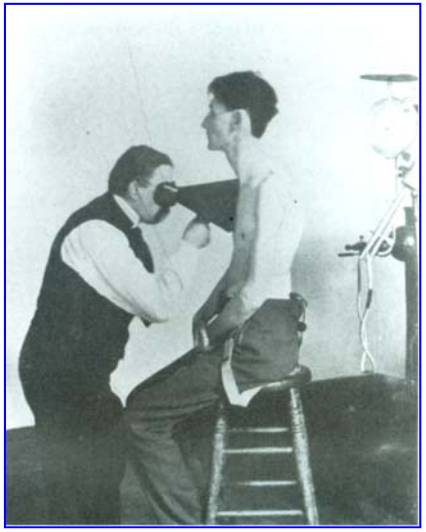
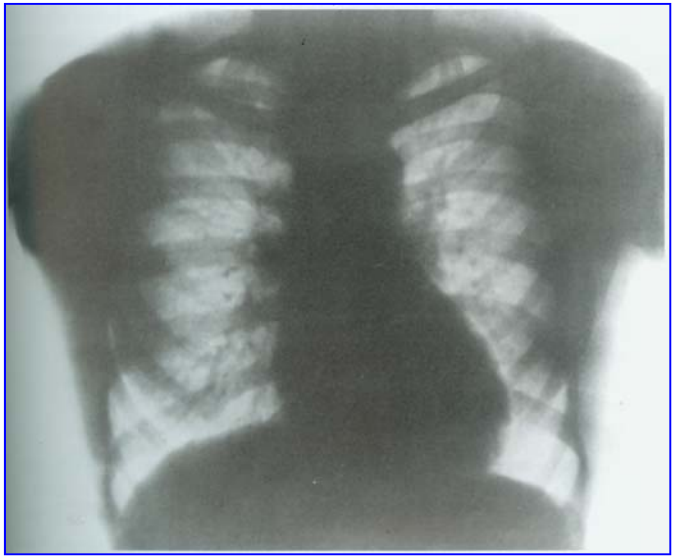
Progress in Radiologic Diagnosis of Tuberculosis 2

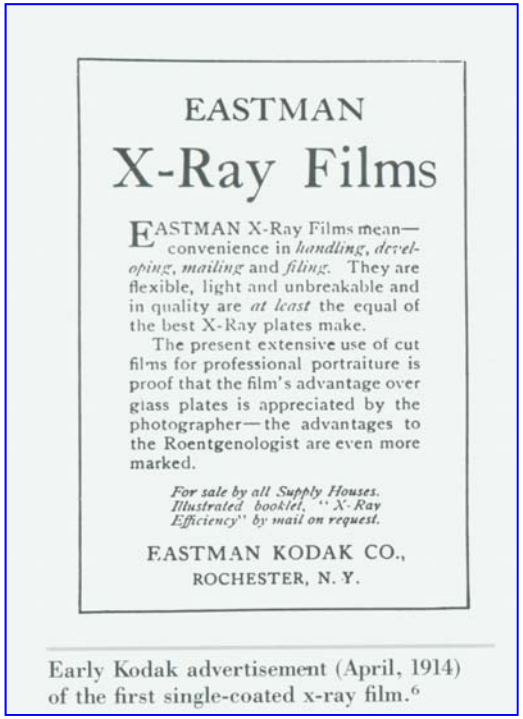
- During the first 30 to 40 years of the 20th century, diagnosis was usually achieved by fluoroscopy without film



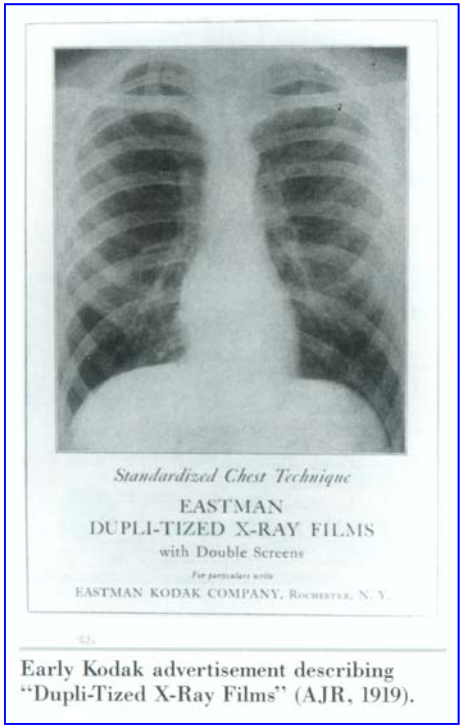


Progress in Radiologic Diagnosis of Tuberculosis 3





Early Kodak advertisement (April, 1914) of the first single-coated x-ray film.[6]

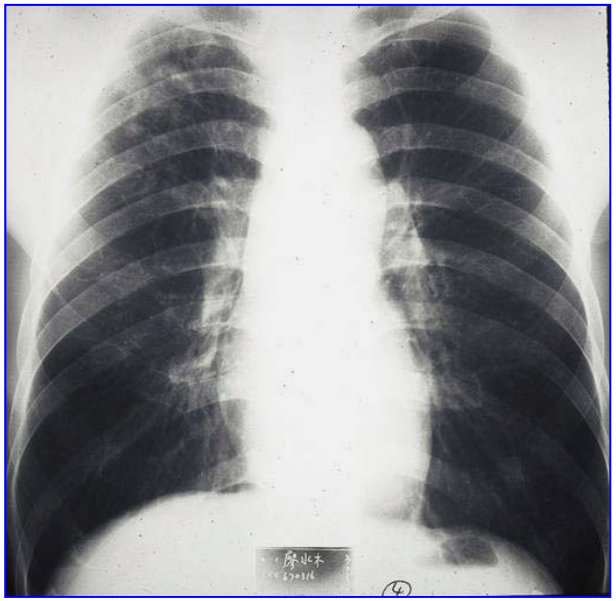


Early Kodak advertisement describing "Dupli-Tized X-Ray Films" (AJR, 1919).

- Images on film were introduced later
- Mass miniature radiography was introduced in the 1940s

Progress in Radiologic Diagnosis of Tuberculosis 4

- Tomography was first used in 1935
- In the early days it was mainly used for chest disease, especially for detecting cavities in areas of TB infiltrations and bronchial narrowing secondary to lung cancer

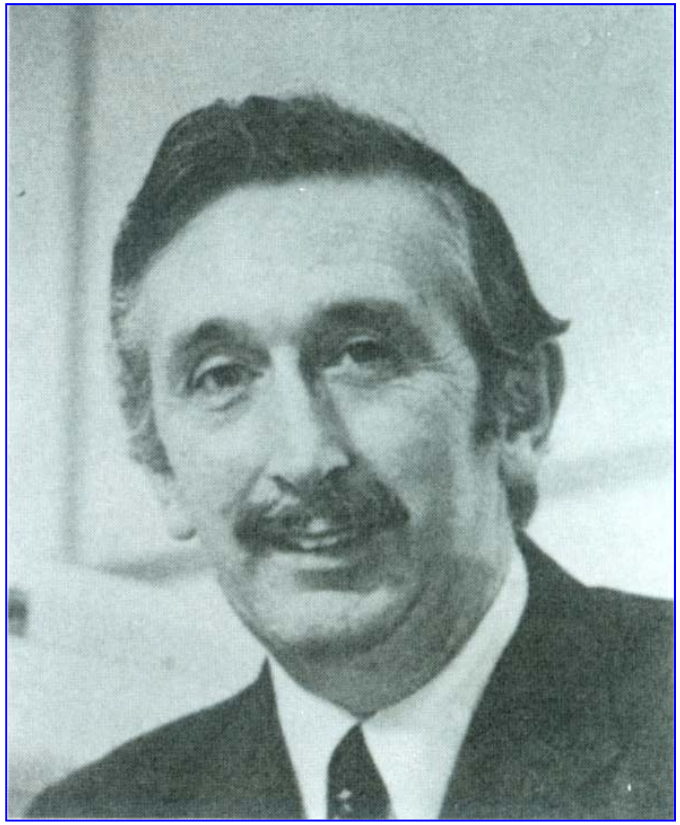


Difficulty in Radiologic Diagnosis of Tuberculosis

- Tuberculosis is a great imitator, may simulate many other diseases
- It may mimic or occur concurrently with pneumoconiosis, sarcoidosis, neoplasms, lung abscess, and fungal infection
- CXR may appear as normal in some cases
- Inter-/Intra- observation variety

Progress in Radiologic Diagnosis of Tuberculosis 4

- Computed tomography was first introduced in 1972 by Godfrey Housefield of EMI Limited in London
- In 1979 he awarded Nobel Prize

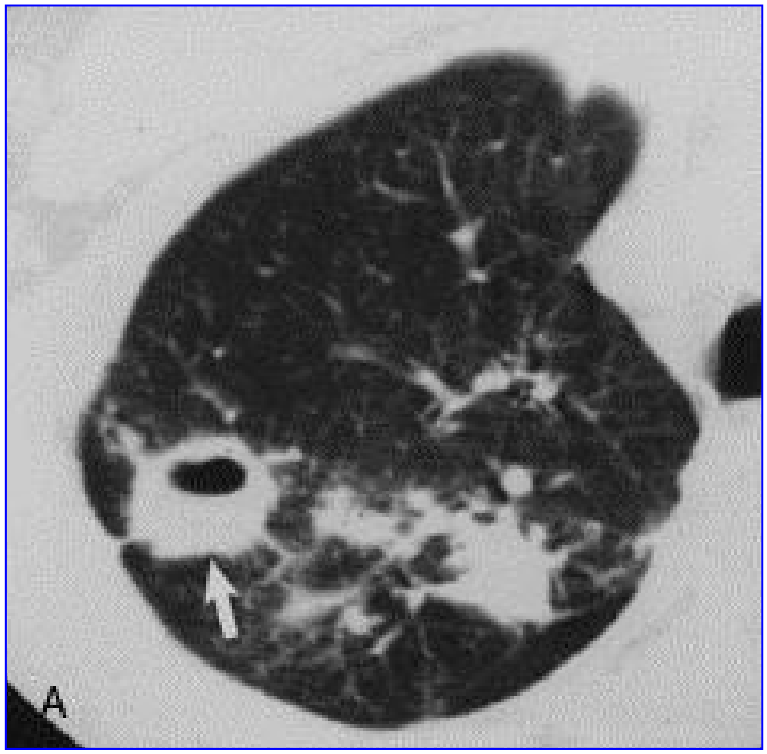


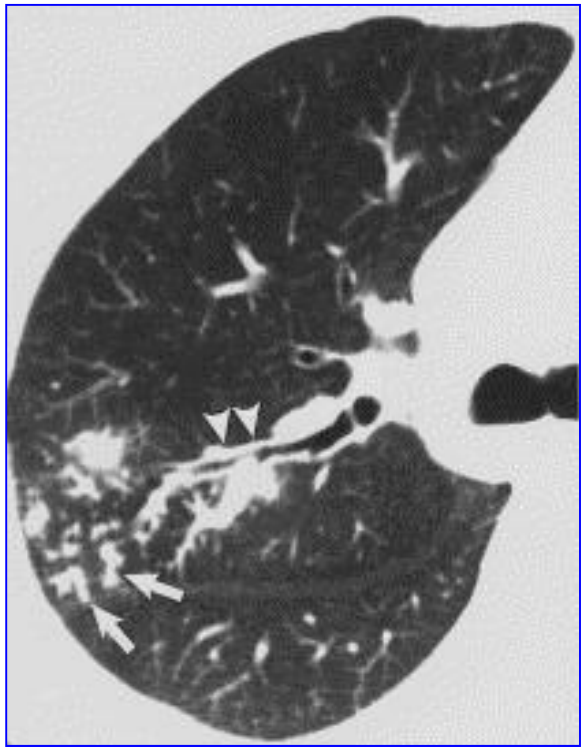
Godfrey Housefield 1919 -

Value of Chest CT in Diagnosis of Pulmonary Tuberculosis

- **Chest CT –**
  to detect fine lesions overlooked on chest PA films,
  to define equivocal lesions, or
  to evaluate complication
- **HRCT (High resolution) –**
  useful in understanding the pathologic process of disease and in determining activity in selected cases

Progress in Radiologic Diagnosis of Tuberculosis 5





Toward the end of the 20th century, CT was valuable to slices of cavities and other lesions, particularly when distinguishing between tuberculoma and cancerous lesions

Conventional Procedures in Mycobacteriology Laboratory

- Collection of specimens
- Specimen preparation
- Acid-fast microscopy
- Isolation by culture
- Identification
- Drug susceptibility testing
- The entire process: 4 to 6 weeks
- Drug susceptibility test: add 3 to 6 weeks

Value of traditional methods for laboratory diagnosis of tuberculosis

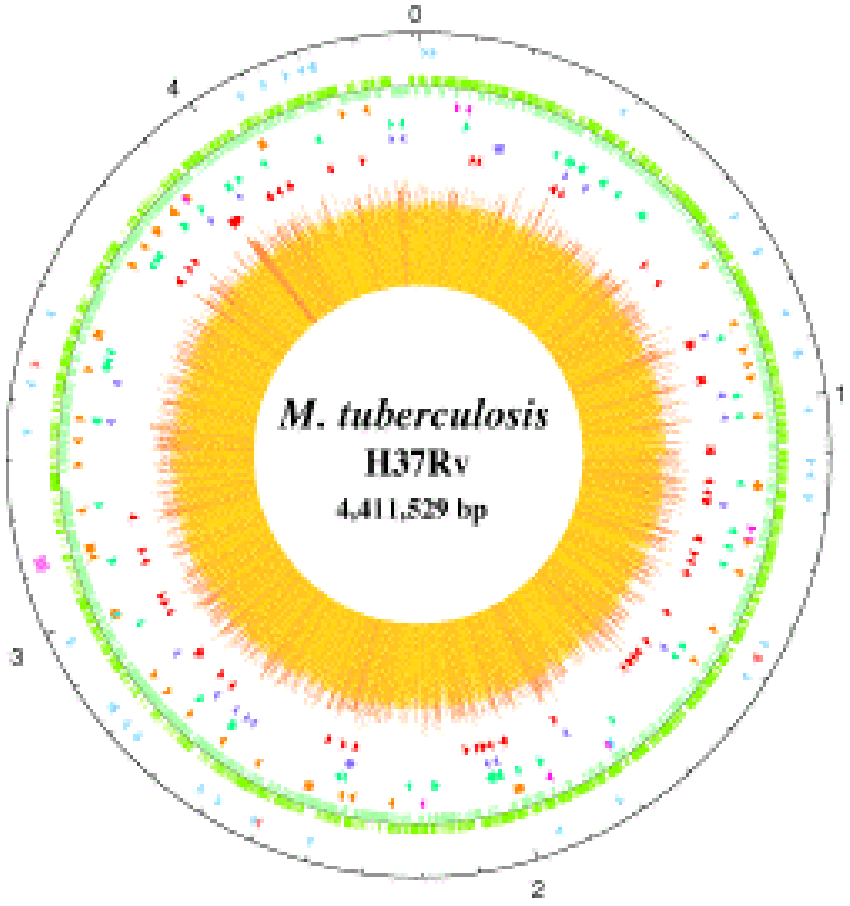
- Although smear microscopy is rapid, its specificity is relatively low (ranging from 8.8% to 46.4% of culture verified cases). Moreover, it cannot reliably distinguish MTB from NTM
- Mycobacterial culture is more sensitive and specific, however, the use of culture is technically challenging and slow, it can take up to 6-8 weeks for MTB growth on culture (solid media)

CDC Recommendations for Standards for Diagnostic Mycobacteriology

- **Provision of AFB smear results within 24 h of specimen collections,**
- **Isolation and identification of *M. tuberculosis* within 10 to 14 days**
- **Provision of susceptibility results within a total of 15 to 30 days**

Huebner RE et al ***J Clin Microbiol*** 1993;31:771-5
Doern GV ***J Clin Microbiol*** 1996; 34:1873-6

Genome of *Mycobacterium tuberculosis* H37Rv

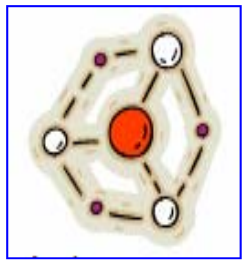


- More than 4.4 million base pairs
- 3924 genes detected initially, 13 more genes uncovered through protemics and compatative genomics
- More than 25 genetic markers identified for typing

Cole ST et al. ***Nature*** 1998; 393: 537.

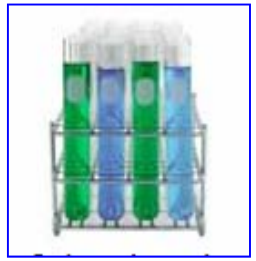
New Approach in Diagnosis of Tuberculosis 1

- Replication of *M. tuberculosis*



1. Antigen detection tests:
   LAM ELISA urinary antigen test,
   sputum antigen test

2. Microscopic visualization of bacteria:
   LED microscopy, bleach microscopy
3. Culture based detection tests:
   Microscopic observation drug susceptibility assay (MODS), thin-layer agar, phage-based tests, calorimetric media



Tests under development

LAM in Urine Chemogen Univ Munich

- ELISA based test
- Detect LAM , antigen 85 (lipoarabinomannan)
- FIND collaboration
- FIND evaluation disappointing

New Approach in Diagnosis of Tuberculosis 2

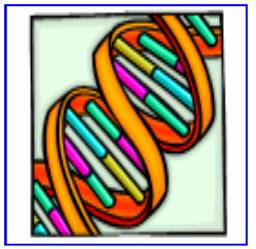
- **Replication of *M. tuberculosis***

4. Nucleic acid amplification tests:

LAMP, Xpert MTB,

Transrenal DNA detection,

Genotype MTBDR*Plus*



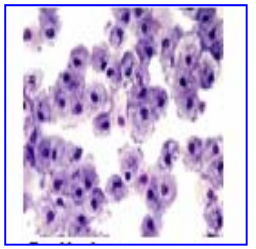
5. Volatile organic compounds (VOC) detection:

E-Nose, biosensors

New Approach in Diagnosis of Tuberculosis 3

- **Immune response to *M. tuberculosis***

1. **Cellular immune response:**
   INF-γ release assays (IGRA)
   Quanti-FELONTB gold,
   T-SPOT TB; rd ESAT-6 skin test



2. **Humoral immune response:**
   Antibody detection tests:
   serological tests

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results

Stop TB Partnership's New Diagnostics Working Group

1. **Sputum collection**
2. **Sputum smear microscopy**
3. **Culture-based methods**
4. **Molecular methods**
5. **Cytokine assay**

Ralph AP et al. ***Clin Infect Dis*** 2009;49:574-83

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results

Sputum Collection

- **Improved sputum-submission guidance**

  If smear (+) pulmonary TB case detection is impaired by poor-quality specimen submission, case detection can be improved by provision of adequate instruction

- **Reduction number of collection from 3 to 2**

  Because increment yields from sputum specimens are small, WHO recommends examining 2 smears; this is can alleviate laboratory workloads, decrease time for diagnosis, and decrease the number of patients who "drop out" of the diagnostic pathway

Ralph AP et al. ***Clin Infect Dis*** 2009;49:574-83

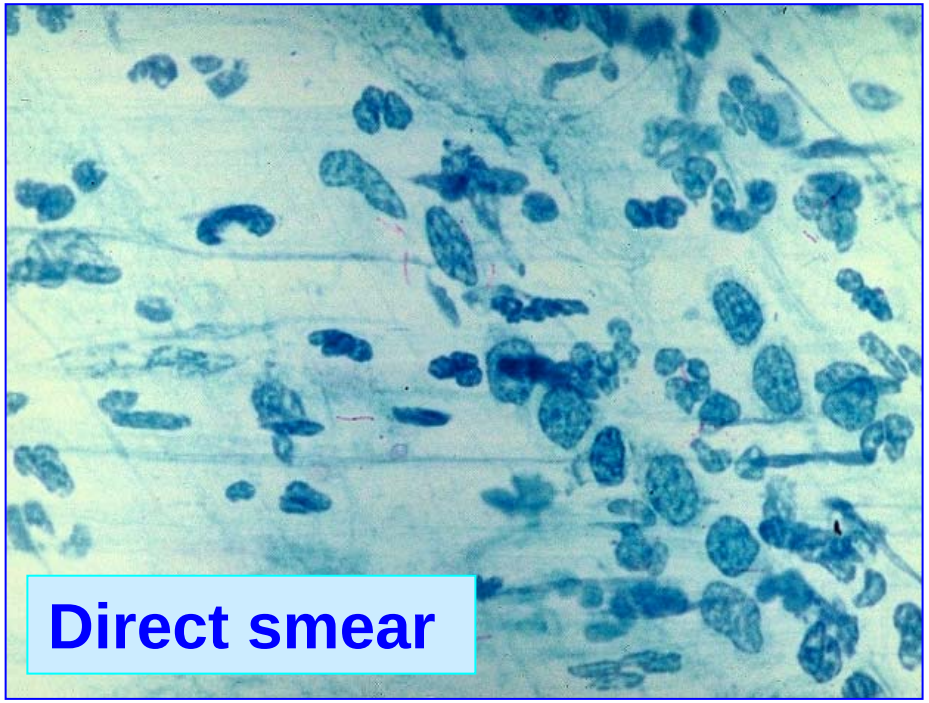
Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Sputum Smear Microscopy 1

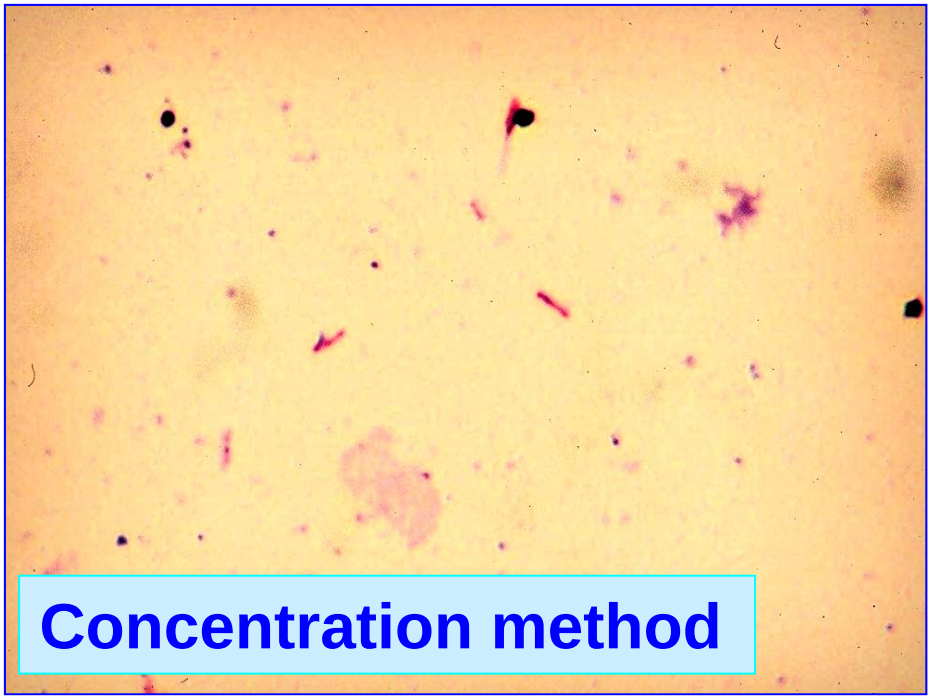
- **Processing of sputum sample prior to smear exam (eg. use of bleach then centrifugation or use of bleach or NaOH then overnight sedimentation)**

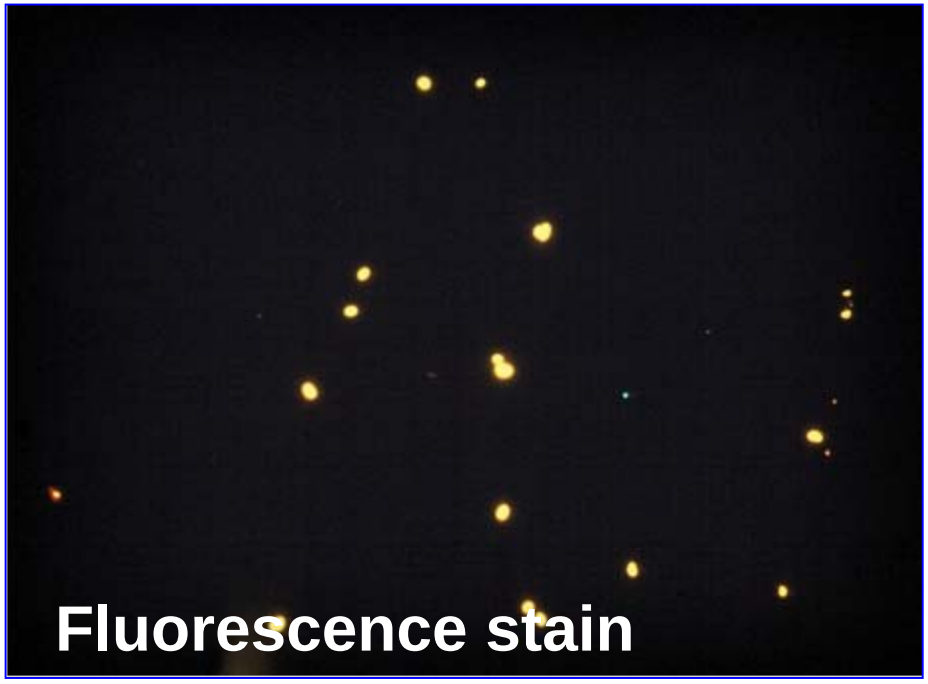
  This is 18%-23% more sensitive than direct microscopy

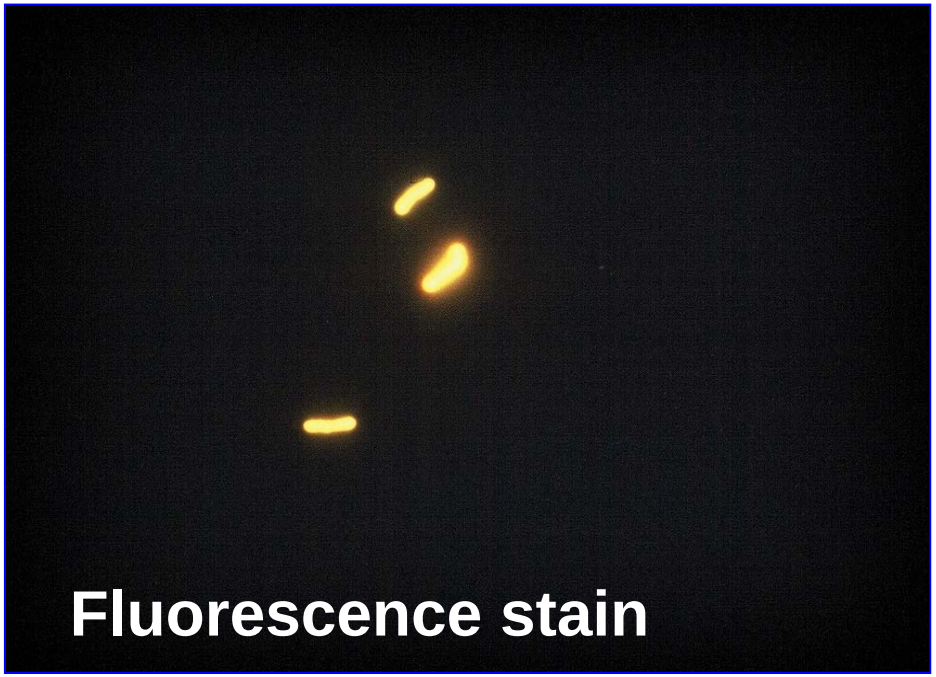
- **Fluorescence microscopy**

  This is 10% more sensitive than conventional microscopy; use to determine viability of *M. tuberculosis* in follow-up sputum specimens to treatment failure


Direct smear


Concentration method

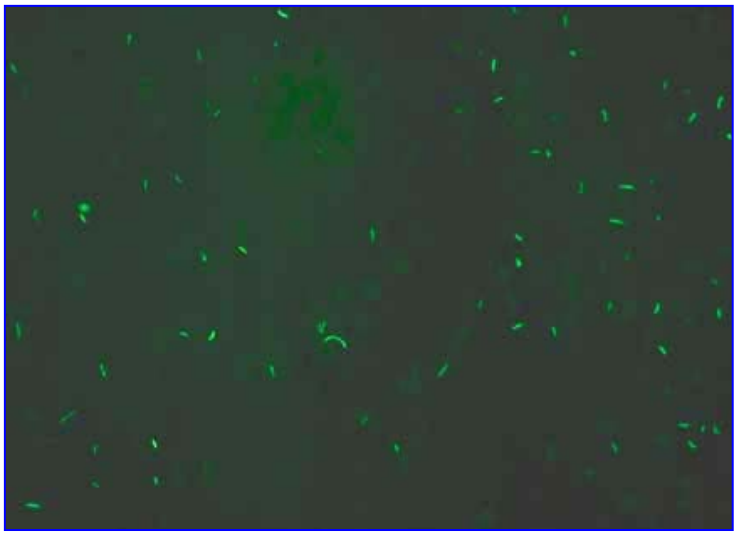

Fluorescence stain


Fluorescence stain

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Sputum Smear Microscopy 2

- **Fluorescence microscopy using light-emitting diode (LED) light source**

  These light source are cheaper, last longer, and have less potential for environmental contamination than do traditional lamp used in this method



From fluorescence to bright field contrast with the flick of a switch

The battery pack makes it possible to work without main power

***From* Carl Zeiss MicroImaging GmbH**

INT J TUBERC LUNG DIS 10(9):1060–1062

TECHNICAL NOTE

Light emitting diodes for auramine O fluorescence microscopic screening of *Mycobacterium tuberculosis*

R. M. Anthony, A. H. J. Kolk, S. Kuijper, P. R. Klatser

Department of Biomedical Research, Royal Tropical Institute (KIT), Amsterdam, The Netherlands

SUMMARY

We describe the simple adaptation of a standard fluorescent microscope for illumination using a 'Royal Blue' Luxeon™ light emitting diode (LED) and demonstrate that this form of illumination is suitable for the detection of auramine O stained *Mycobacterium* spp. The low cost, low power consumption, safety and reliability of LEDs makes them attractive alternatives to mercury vapour lamps.

KEY WORDS: fluorescence; microscope; LED; auramine

Smear Microscopy Improvement

- **New LED Microscopes**
  - **FluoLED**
  - **Zeiss Primo Star iLED**
- **Evaluation of FluoLED module**
  - **461 smears by FluoLED and Conventional fluorescence and ZN method**
  - **99% concordance between two fluorescence methods**
  - **Increase sensitivity as compared to ZN**

Van Deun et al ***Int J Tubercul Lung Dis*** 2008, 12:1014

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Culture-based Methods 1

- **Liquid culture (eg. automated mycobacteria growth indicator tube)**

  Faster and more sensitive than solid media; recommended standard method

- **Microscopic observation drug susceptibility assay**

  Yields faster culture and DST results than do liquid or solid media and is inexpensive, but requires skilled technician to interpret culture appearance of *M. tuberculosis*

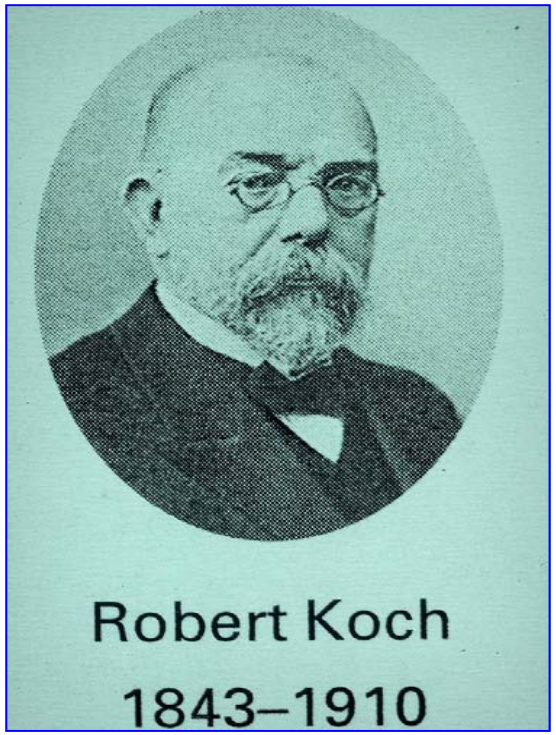
Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Culture-based Methods 2

- **Thin-layer agar methodology**

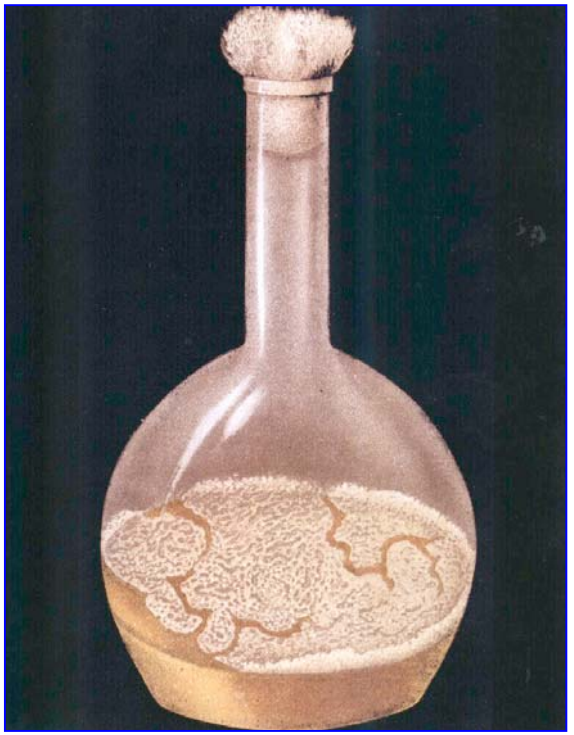
  Yields faster culture and DST results than do liquid or solid media and is inexpensive, but requires skilled technician to recognize *M. tuberculosis* colony formation

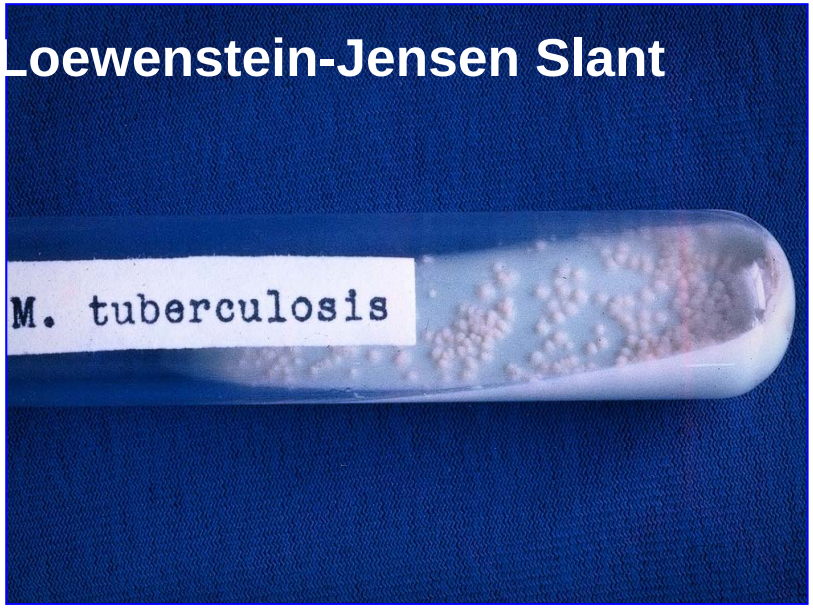
- **Calorimetric DST methods using redox tetrazolium salts, or a nitrate reductase assay**

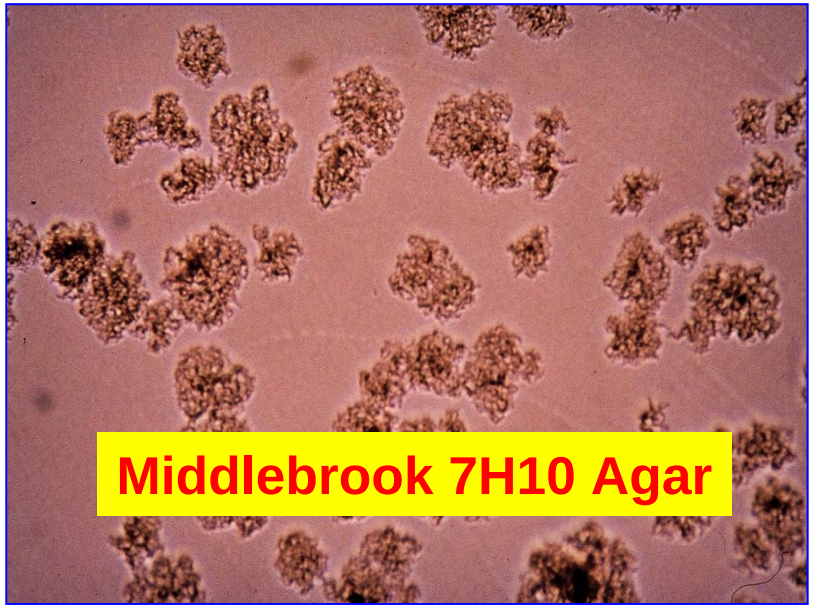
  These are lower cost, low-tech, and able to yield DST results within 2 weeks; potential for biosafety hazard


Robert Koch
1843–1910

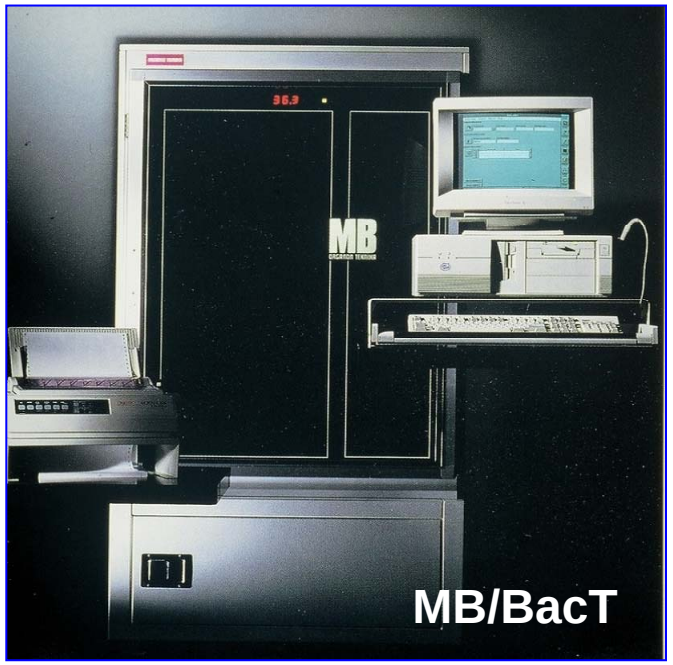

ROBERT KOCH
柯霍氏發現結核菌百週年紀念
2
中華民國郵票
REPUBLIC OF CHINA

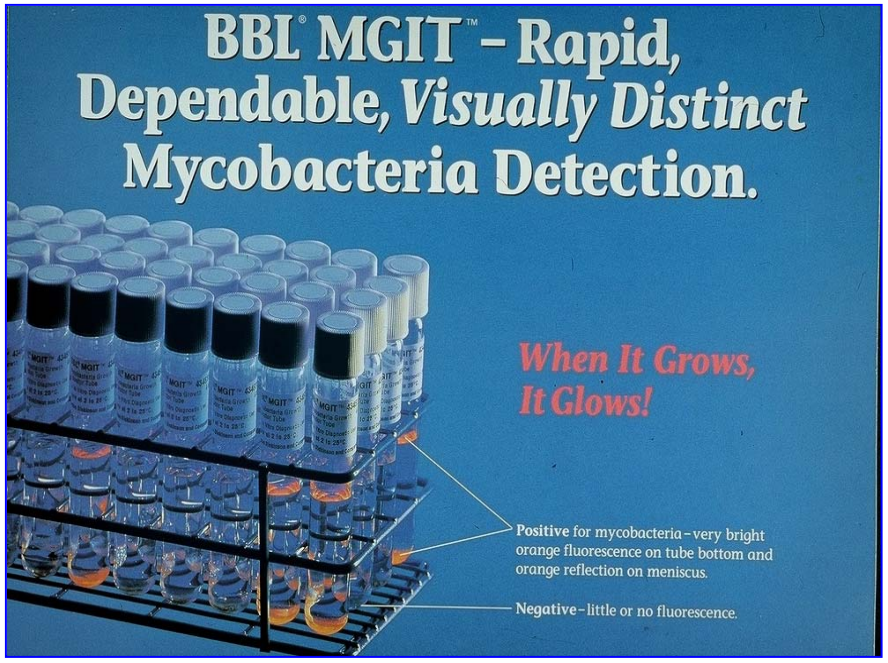



Loewenstein-Jensen Slant
M. tuberculosis


Middlebrook 7H10 Agar

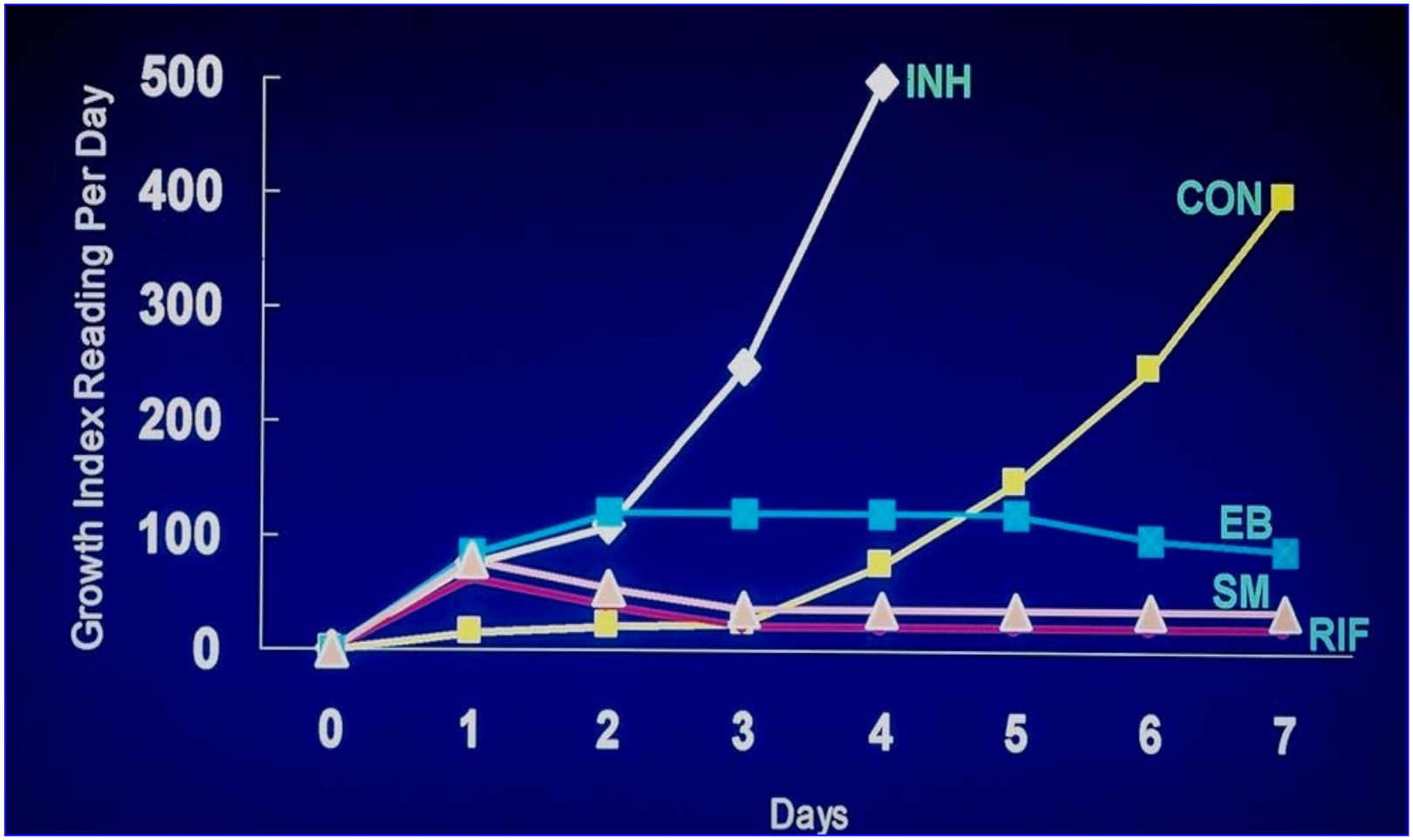

BACTEC 460
BACTEC 460
SAMPLE
GROWTH
GROWTH DETECTION
OPERATING CONTROLS

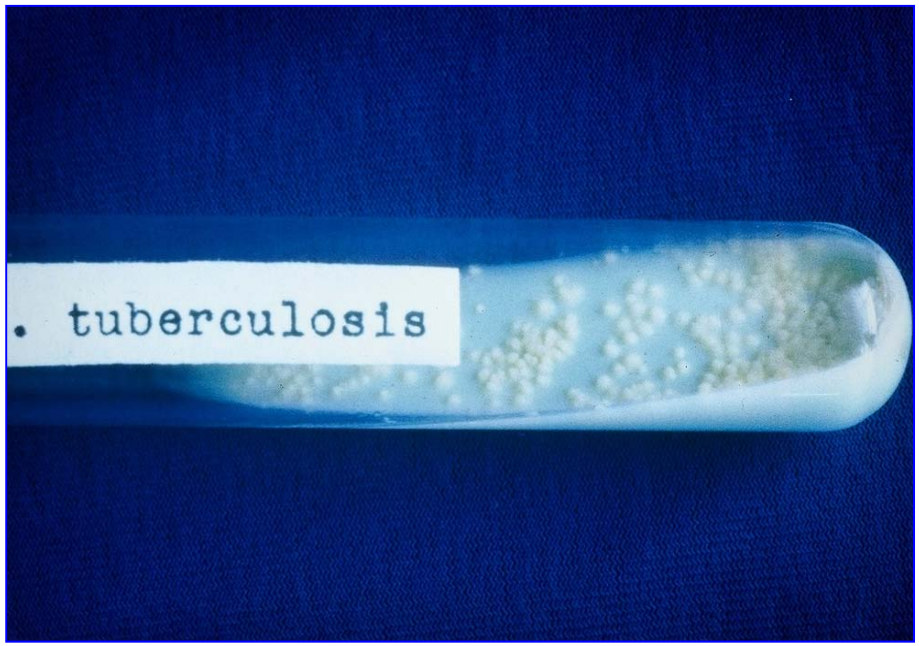

MB
MB/BacT

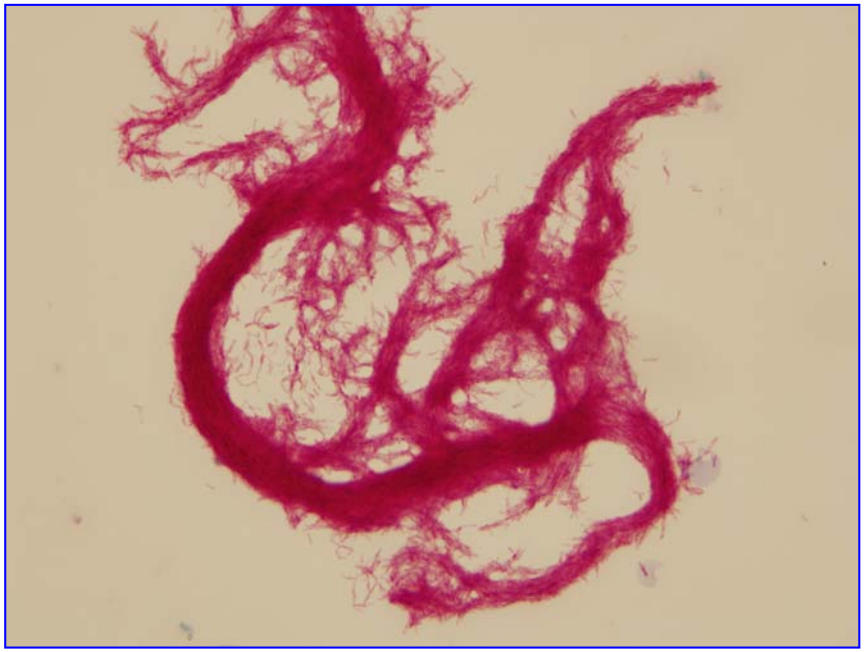

BBL® MGIT™ – Rapid, Dependable, Visually Distinct Mycobacteria Detection.
When It Grows, It Glows!
Positive for mycobacteria – very bright orange fluorescence on tube bottom and orange reflection on meniscus.
Negative – little or no fluorescence.


BACTEC, MGIT 960

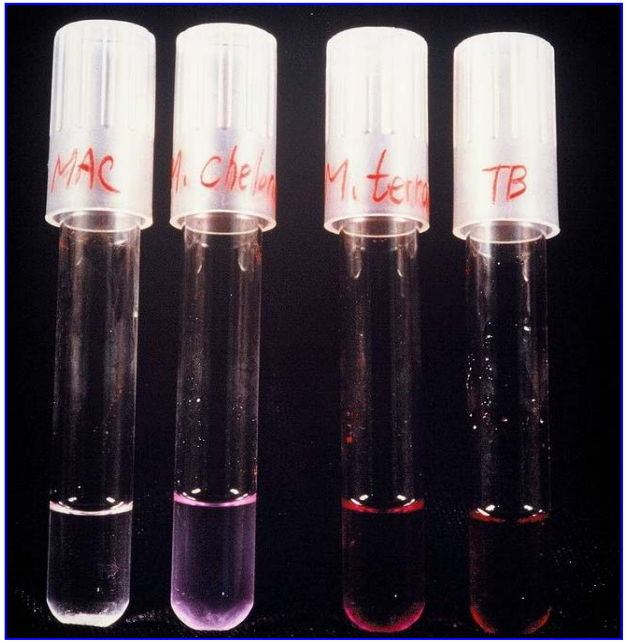
BACTEC Drug Susceptibility Testing of *M. tuberculosis*




. tuberculosis




Neg. Control
M. chelonae
M. marinum
M. simiae
TB +


MAC
TB

抗原偵測

檢體: **MGIT or LJ elution**

- BD Capilia TB
  - Antigen: MPB64 (MTB64) immunochromatographic assay (ICA)

檢體: **Serum, Plasma, CSF, PE,….**

- 台塑結核菌抗原快速檢驗試劑
  - Antigen: CFP10-ESAT

GenoType® Product Series Mycobacteria

Based on DNA•STRIP® Technology

CE-labelling

Quality management certified to ISO 9001

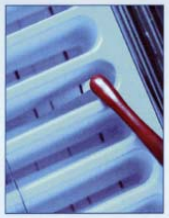
Compliant with the European IVD Directive

HAIN LIFESCIENCE

Info: www.hain-lifescience.de

GenoType® Mycobacterium CM
GenoType® Mycobacterium AS

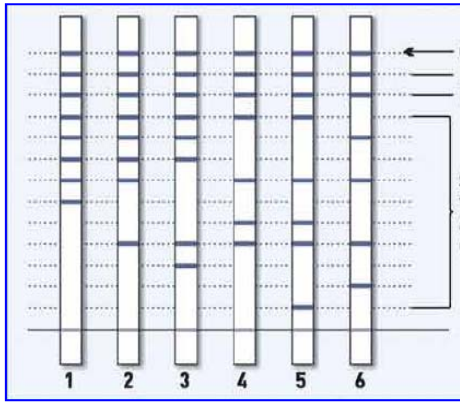
Molecular Genetic Test Systems for the Differentiation of Mycobacteria from Culture Specimens



- simple
- safe
- fast
- easy to combine
- can be automated

CE-labelling

Quality management certified to ISO 9001



Specific banding patterns of GenoType® MTBC

1. *M. tuberculosis*
2. *M. africanum I*
3. *M. microti*
4. *M. bovis ssp. bovis*
5. BCG
6. *M. bovis ssp. caprae*

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Molecular Methods 1

- **Nucleic acid amplification tests (NAATs)**
  - **High specificity and positive predictive value, important role in confirming mycobacterial identification; but poor negative predictive value for pulmonary and extra-pulmonary TB; updated US CDC guidelines recommend sputum *M. tuberculosis* NAATs for cases of suspected, unconfirmed TB if results would alter management**
  - **In-house ("home-brew") NAATs produce highly inconsistent results as compared to commercial, standardized NAATs**

Automated AMPLICOR™ Testing from the Leader in PCR Diagnostics

Molecular Diagnosis

GEN-PROBE
Pioneering Advances In Healthcare Through DNA Probes

DIGENE

AFFYMETRIX

BD

Roche

VYSIS

CHIRON

AsiaGen Corporation
亞洲基因科技股份有限公司

Nest-PCR Assay

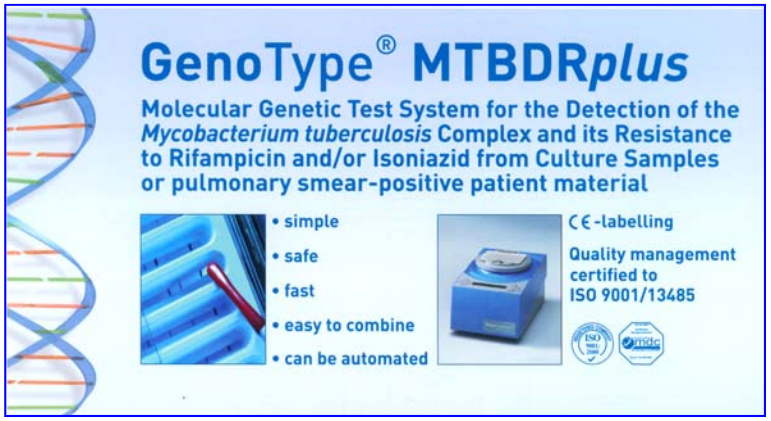
Dr. Chip Corp.

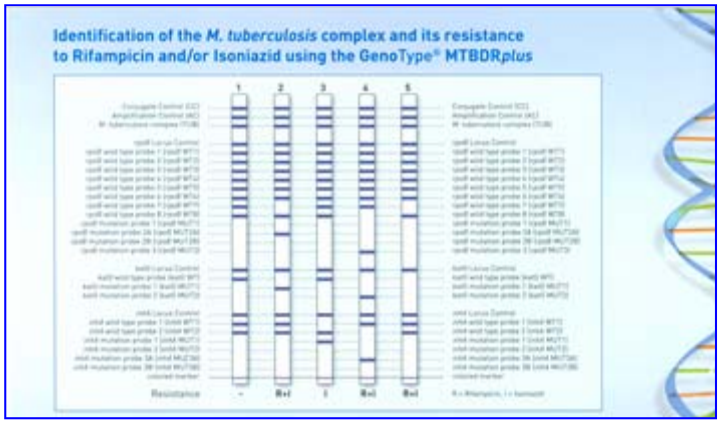
Dr. MTBC Screen Assay

Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results
Molecular Methods 2

- **Line probe assays (eg. Genotype MTBDR*plus* assay [Hain] and INNO-LiPA Rif.TB assay [Immunogenetics]**

High sensitivity and specificity for detection of rifampicin (with or without INH) resistance, with a 1-2 day turn around time directly for smear positive sputum; requires DNA extraction and amplification facilities





WHO Recommendations

Line Probe Assays

- **Rapid screening of High Risk patients for detection of MDR TB**
- **Only on Smear-positive specimens or isolated cultures**
- **Use commercially available tests**
- **Does not eliminate the need for Culture and DST capability**
- **Need at least BSL 2 Lab with BSC**
- **Require at least three separate rooms**

WHO Policy Statement, June 2008

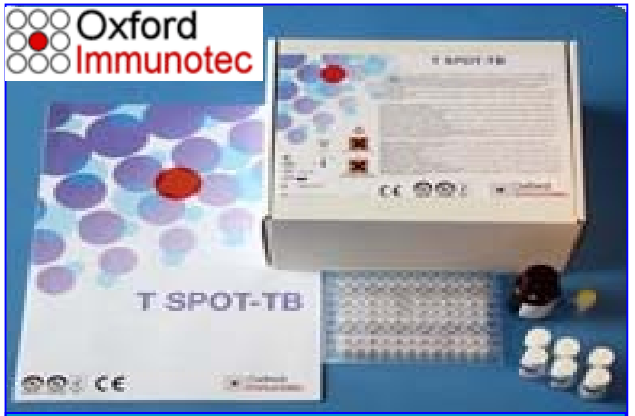
Methods to Improve Diagnosis and Accelerate Drug Susceptibility Results

Cytokine Assay

- **T cell interferon-γ release assay (IGRA)**

Useful in targeted strategy for latent TB infection (LTBI) detection in low TB-incidence settings;
more specific than tuberculin skin test;
cannot distinguish active from treated TB or LTBI

Antigens: ESAT6-CFP10
IFN-γ by ELISA



Antigens: RD1 protein
IFN-γ by **ELISPOT**

World Health Organization

Diagnostics Evaluation Series
No. 2

Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis

Special Programme for Research & Training in Tropical Diseases (TDR) sponsored by
UNICEF/UNDP/World Bank/WHO

New Methods New Tests Where do these stand

- Microtiter Plate for DST – No hope
- Micro Colonies (MODS) - Struggling
- E-Test (AB Biodisk) - Gone
- Phage-Based – Gone
- BD MGIT System – Holding well
- Biomeureoux
  - DST – Gone
  - Isolation – Phasing out
- Other Liquid Media - Gone
- Molecular – Holding well, newer tests have big potential, but with limitations

Tests under development

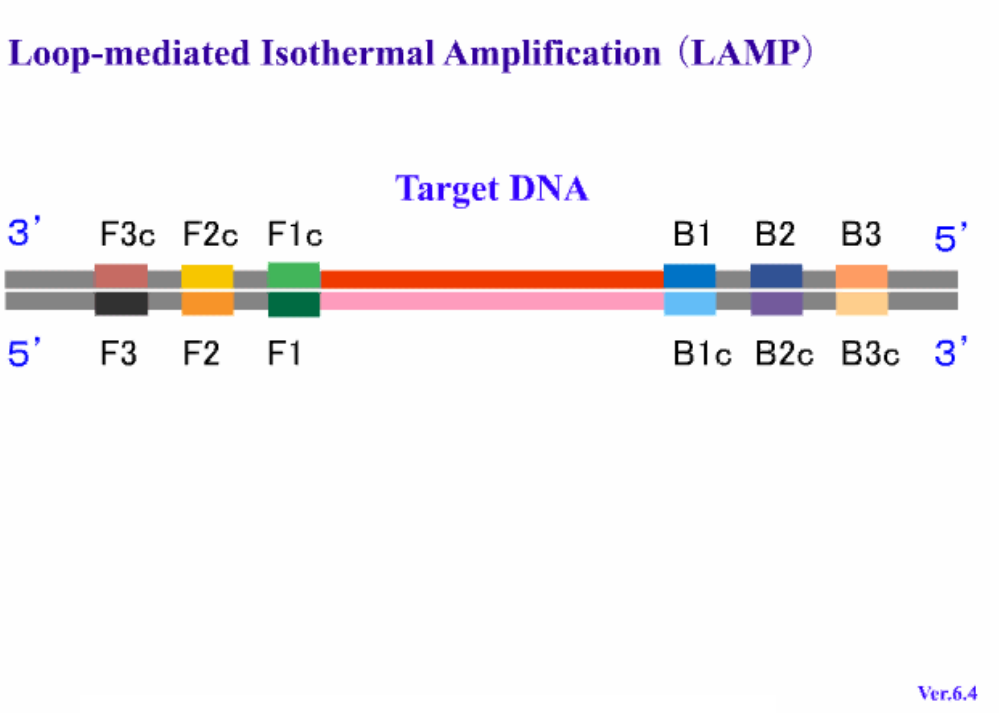
Eiken, Japan

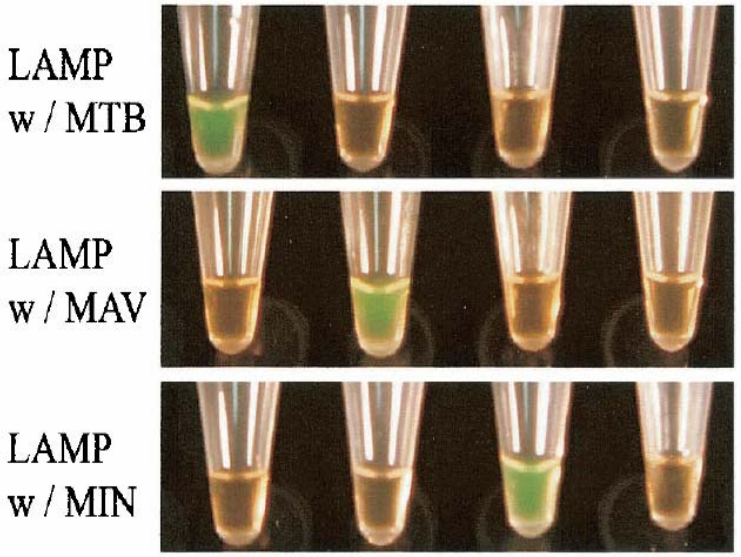
- **Loop mediated isothermal Amplification (LAMP) of DNA**
  - **Small heating device**
  - **Runs at high temp (avoids non-specific amplification)**
  - **Multiple primers sets (increased specificity and speed)**
- **Direct from Sputum**
- **Closed System** No risk of contamination
- **Minimal Instrumentation**
- **Fast** Less than 2 hours total
- **Visual detection.** No instrumentation
  - $Mg_2P_2O_7$ ppt detection (white). Other colors possible
- **Specimen Processing ???**
- **FIND Collaboration studies look promising**

IUATLD, 2008

Simple, manual NAAT

EIKEN CHEMICAL CO.,LTD.





- Closed system
- Isothermal
- Rapid
- Multiprimer
- Visible readout

MTB / Rif-resistance 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

cartridge

Strategic & Technical Advisory Group for TB (STAG-TB) and WHO recommendations

"STAG-TB endorsees the WHO recommendations for the use of liquid cultures and rapid species identification to address the needs for culture and drug susceptibility testing (DST), integrated in a country specific comprehensive plan for laboratory strengthening"

MMWR-Feb 13, 2009
CDC Reports and Recommendations

Diagnostic Laboratory

- The laboratory plays a critical role in the diagnosis and management of drug-resistant TB.
- **Test results must be available in a time frame that allows clinicians to make prompt patient management decisions.**
- Many laboratory techniques used to confirm a TB diagnosis and to identify drug resistance were developed in the 1950s, 1960s, and 1970s.
- Substantial improvements have been made in culture techniques and in rapid methods in the past decade.

MMWR-Feb 13, 2009
CDC Reports and Recommendations

- However, these more accurate, rapid, and sophisticated methods have not been implemented widely, particularly in regions of the world where MDR TB and XDR TB are common and optimized algorithms for providing rapid point-of-care laboratory confirmation of TB and detection of drug resistance have not been established.
- To combat the growing problem of resistance to TB drugs, the **most current methods need to be applied to their fullest capacity** while better diagnostic tests are developed.
- **The needs of the TB laboratory must be addressed to make laboratory services for TB, MDR TB, and XDR TB more rapid, sensitive, reliable,** and more responsive to the needs for patient management, infection control, and TB control efforts.

Conclusion

- **Newer technologies offer a significant time savings. However, these tests have limitations**
  - **Costly**
  - **Complex and cumbersome**
  - **Only smear-positive (50% of culture-positive)**
  - **Recommended only in special cases**
  - **Add-on tests**
- **Culture is still the Gold Standard. As recommended by CDC/WHO**
  - **Whenever possible, use liquid culture and DST**
  - **Rapid testing and reporting essential for TB control**


Thank You !