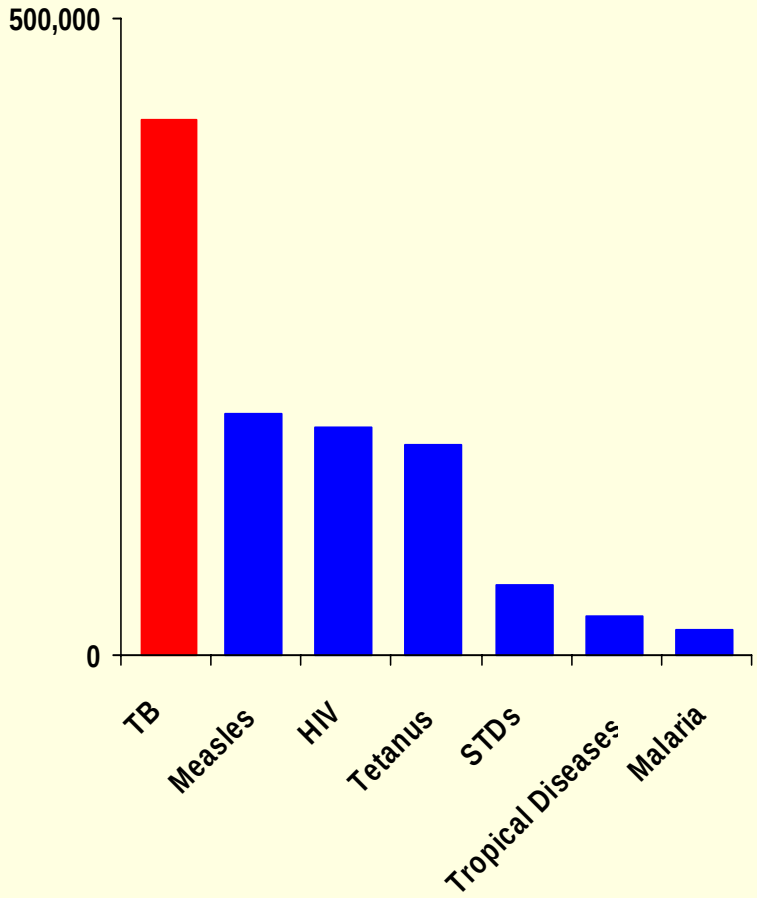
New Modalities in TB Diagnosis

Introduction

- Presently, about 1/3rd of the world's population is infected with Mycobacterium tuberculosis (M.tb).
- It is estimated that currently there are about 10 million new cases of TB every year with 3 million deaths occurring world-wide.*
- Death from TB comprise 25% of all avoidable deaths in developing countries.

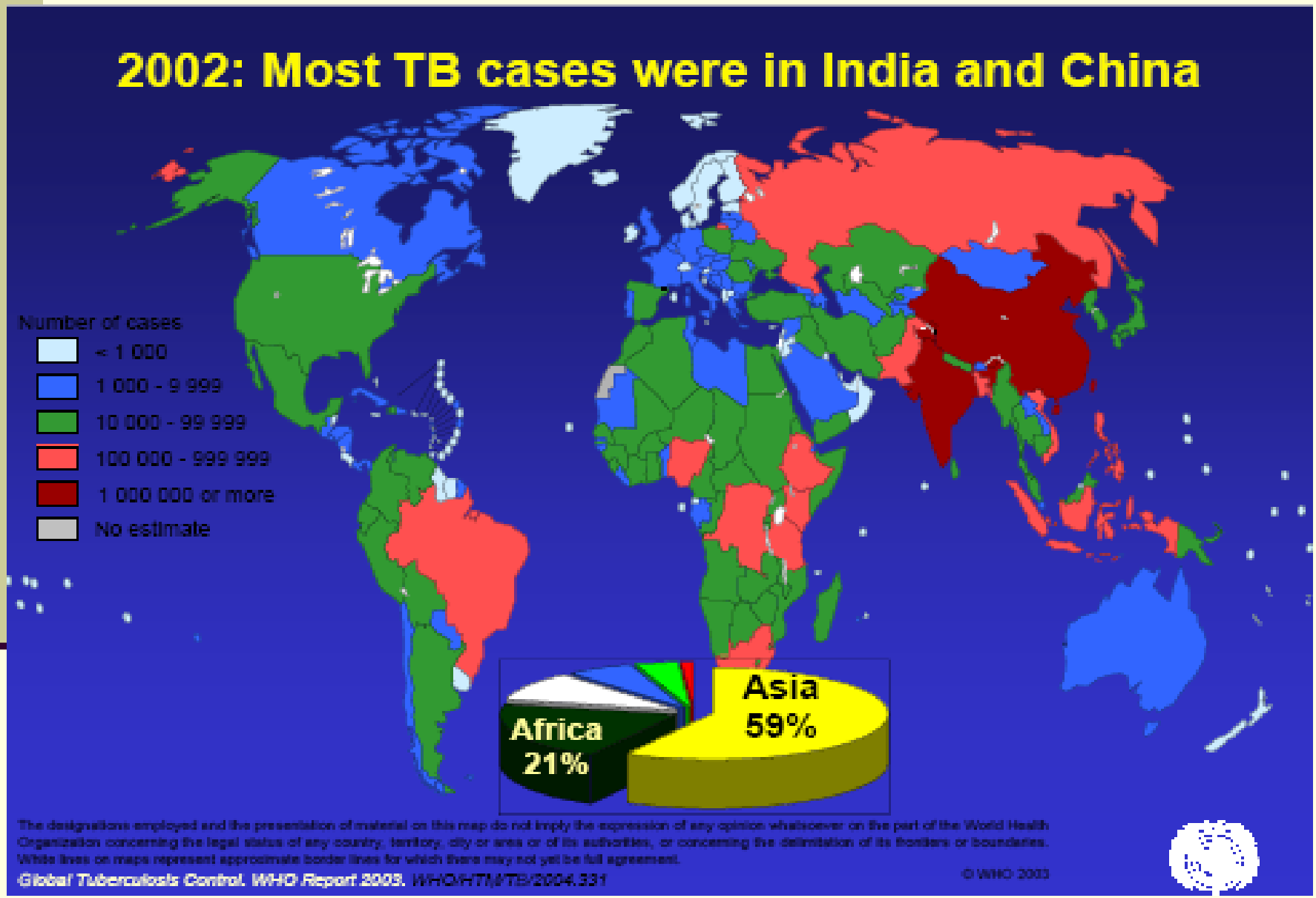
**JAMA 1999;282: 677*

- In India, out of a total population of over 1 billion, each year about 2 million develop active disease and up to half a million die.*
- It implies that every minute, a death occurs due to TB in our country.



WHO/TB/97 1997;231: 998

2002: Most TB cases were in India and China

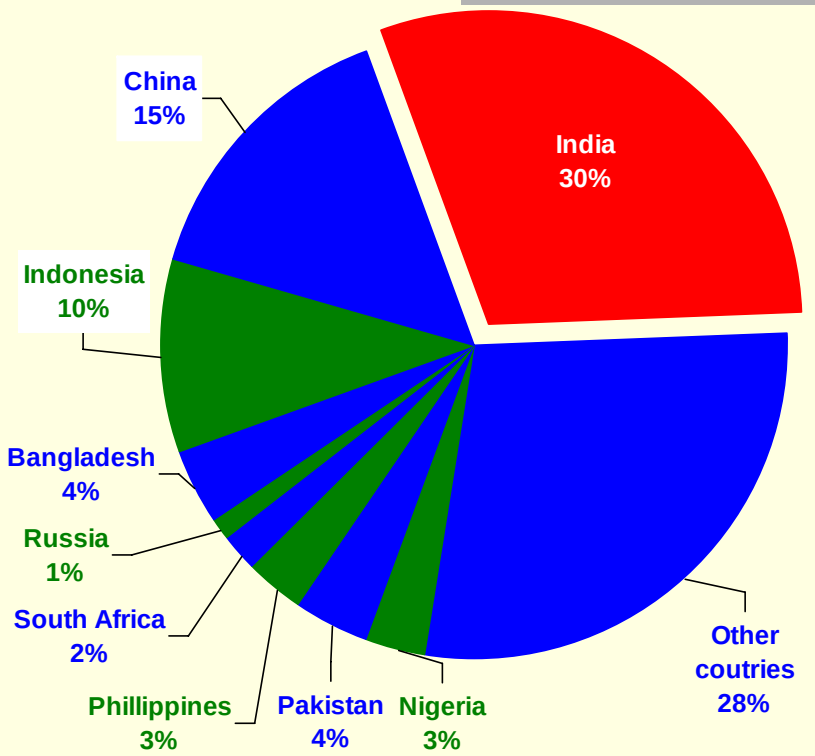


The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. White lines on maps represent approximate border lines for which there may not yet be full agreement.

Global Tuberculosis Control. WHO Report 2003. WHO/HTM/TB/2004.331

- Early diagnosis of TB and initiating optimal treatment would not only enable a cure of an individual patient but also will curb the transmission of infection and disease to others in the community.**

Ind J Tub 1995; 42: 95

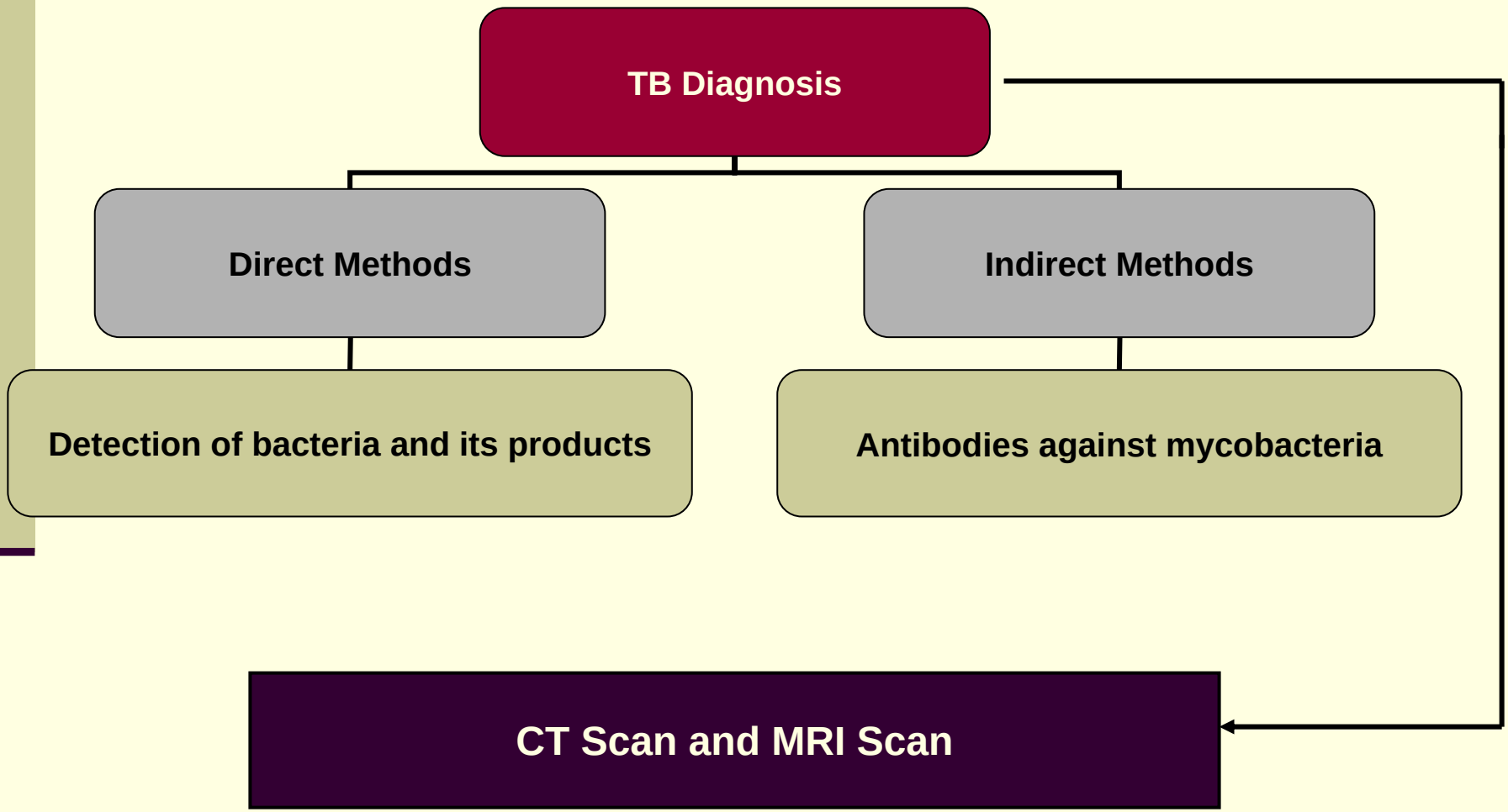


- The diagnostic needs in disease non-endemic countries include:
  - Identification of latent infection in high risk group.
  - Diagnosis of patients in early phase of disease.
  - Faster detection of outbreaks.
  - Finding out patients with non-tuberculous mycobacterial disease.

- The diagnostic needs in disease endemic countries include:
  - Improved microscopy.
  - Usage of liquid culture for childhood and extra pulmonary TB.
  - Chemical and physical detection of mycobacterial antigens in paucibacillary condition.
  - Antigen capture, Antibody detection and cellular immune recognition.

- The diagnosis in endemic countries depends more on the use of labour intensive, easy to use methodology with minimum infrastructure or equipment.
- The need is to find a viable alternative for smear microscopy.
- This method has to have these desirable features :
  - Results within 2 hours.
  - Simple training.
  - Easy interpretation.
  - Should function well in HIV +ve patients.
  - Should allow start of treatment as early as possible.

Methods


TB Diagnosis
Direct Methods
Indirect Methods
Detection of bacteria and its products
Antibodies against mycobacteria
CT Scan and MRI Scan

Direct Methods

- **Direct Microscopy** (ZN, Kinyoun, Flurochrome).
- **Culture** (Traditional, Rapid methods).
- Detection of DNA or RNA of mycobacterial origin
  (PCR, LAMP, TAA / NAA, LCR, Fast Plaque).

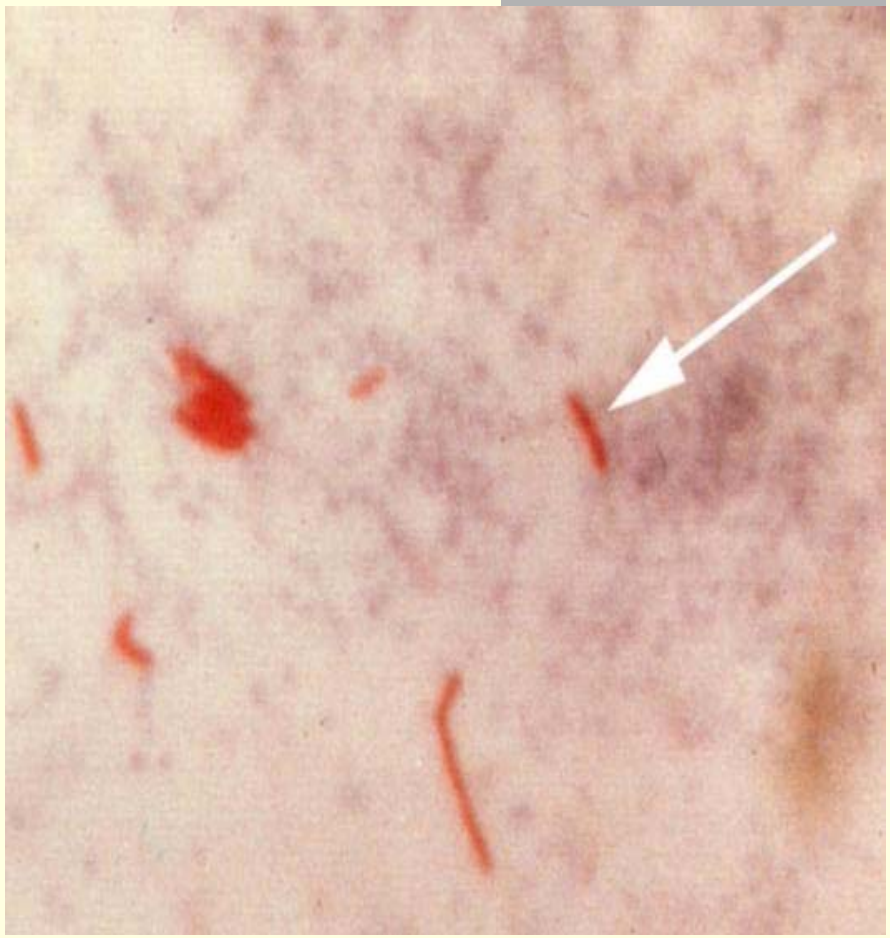
Direct Microscopic Examination

- Hallmark of staining is Ziehl-Neelsen stained slides.
- Easiest & quickest diagnostic test.
- Limited sensitivity (46-78%) but specificity is virtually 100%.
- Centrifugation & flurochrome staining (auramine O) with UV microscopy markedly increase the sensitivity & a large number can be examined in a much shorter time.*

Chest 1969;95:1193

Direct Microscopic Examination

- ZN staining requires = 10^5 bacilli/ml.
- TB bacilli appear as straight/curved rods (1-4μ x 0.2-0.8μ) singly, in pairs or in clumps.
- The yield of microscopic examination correlates well with the extent of disease, the presence of cavitation, and the quality of specimen.
- It is a good marker for infectiousness & the response to the treatment.

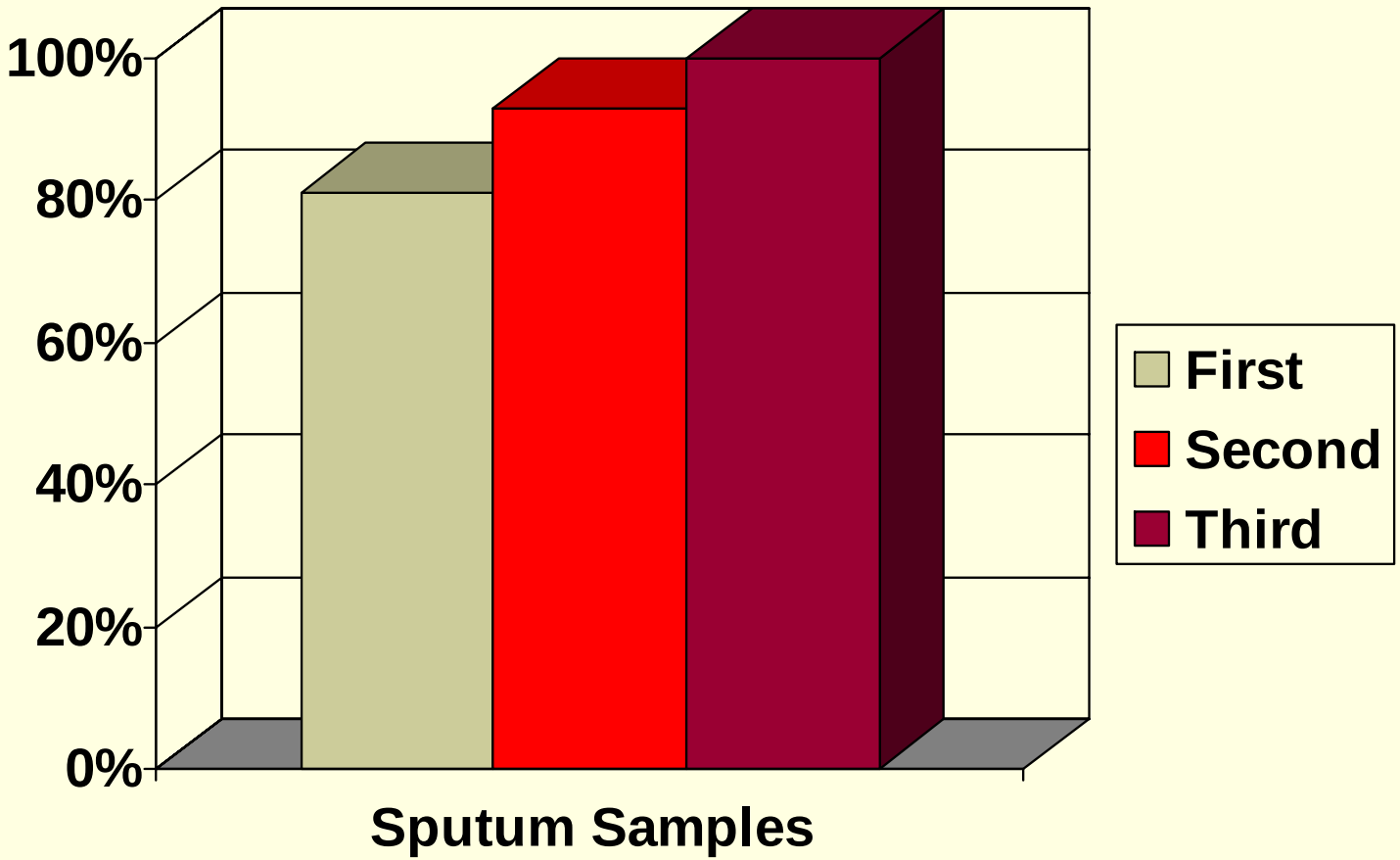


- Several approaches are being made to enhance the sensitivity of the smear microscopy :
  - Concentration of sputum sample by centrifugation enhances sensitivity to almost 100%.*
  - Treatment of sputum samples with Zwitterionic detergent, also known as C_{18} carboxy-prophylbetaine(CB18) interferes with the innate buoyancy of the bacilli and enhances the result of sputum microscopy.**

J Clin Microbiol 1999;31: 2371

****J Clin Microbiol 1998;36: 1965***

Multiple sputum sampling



Traditional Culture

- More sensitive & can be positive even when bacterial load is low (10-100 bacilli/ml).
- Sensitivity 80-85%; Specificity 98%.
- Required for precise identification of causative organisms.
- 3 Types of media are used:
  - Egg based: LJ, Petragnani and ATS.
  - Agar based: Middlebrook 7H10 or 7H11.
  - Liquid based: Kirschner's, Middlebrook 7H9.
- Growth is slow and takes 6-8 weeks . There after the same length of time is required for complete identification & sensitivity testing.

Traditional Culture

Broth Based Rapid Culture Methods

- Micro colony detection on solid media.
- Radiometric (BACTEC).
- Septicheck AFB.
- Mycobacterial growth indicator tubes (MGIT).

- *Substantial improvement in time to detection & total number of positive cultures can be realized from using broth based systems.*

Micro colony Detection on Solid Media

- Plates poured with thin layer of Middlebrook 7H11 agar medium are incubated and examined microscopically on alternate days for the first 2 days and less frequently thereafter.

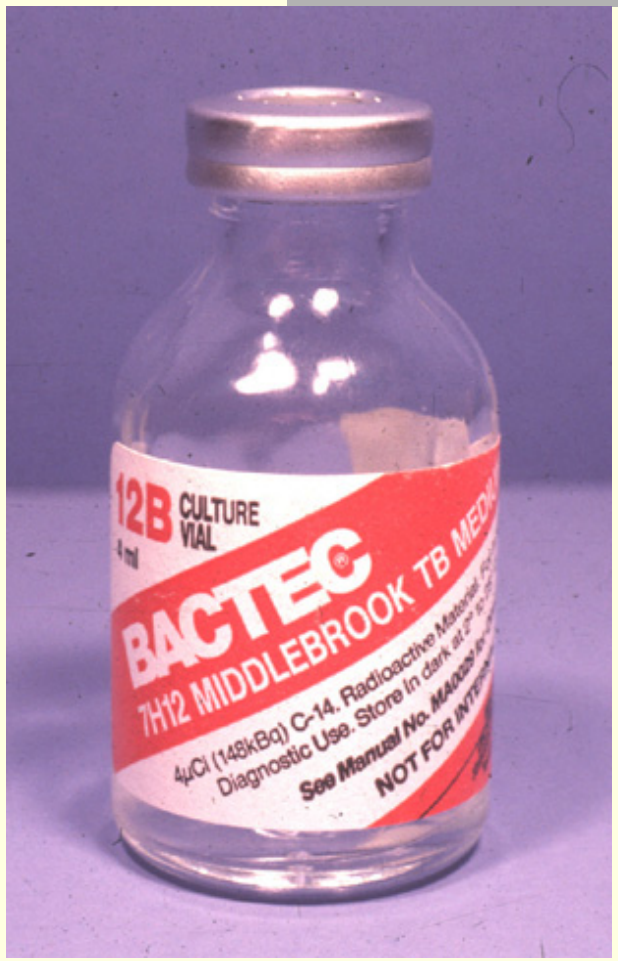
- In less than 7 days micro-colonies of slow growing mycobacteria such as M.tb can be detected.

BACTEC

- Radiometric method. *(Cummings 1975)*
- 4 ml of Middlebrook 7H12 broth containing carbon-14 labeled palmitic acid is used.
- 0.5 ml of processed specimen is added along with a mixture of antibiotics to the broth.

BACTEC

- Growth is ascertained by liberation of $14CO_2$ as metabolized by mycobacteria & detected by BACTEC 460 instrument & reported in terms of growth index (GI) value.



BACTEC

- Average time to recovery of M.tb from smear positive specimens is 8 days.
- When smear negative, culture positive samples are examined, mean time for detection is 14 days.
- More sensitive than traditional method.*
- Can also be used for drug susceptibility testing.

J Clin Microbiol 1994;32: 918-925

BACTEC

- A special procedure unique to BACTEC system for identification of M.tb complex is based on observation that p-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) will inhibit organisms belonging to M.tb complex while having little or no effects on other mycobacteria.

- Drawbacks :
  - Cost.
  - Problem of disposal of radioactive waste.

BACTEC

BACTEC 460

BACTEC 900MB

Septicheck AFB

- Combines broth & solid media into a single device (biphasic culture approach).
- Contains 30ml of modified Middlebrook 7H9 broth in CO_2 enriched culture bottle & a peddle with agar media- one side of peddle covered with Middlebrook 7H11; other side contains Middle brook 7H11 with NAP.
- Requires 3 weeks of incubation
- Advantage: Simultaneous detection of Mtb. NTM, other respiratory pathogen & even contaminant.

Mycobacterial Growth Indicator Tube (MGIT)

- Rapid Method.
- Consists of round bottom tubes containing 4 ml of modified Middlebrook 7H9 broth which has an oxygen sensitive fluroscent sensor at the bottom.*
- When mycobacteria grow, they deplete the dissolved oxygen in the broth & allow the indicator to fluoresce brightly in a 365nm UV light.

** J Clin Microbiol 1999;37: 748-752*

Mycobacterial Growth Indicator Tube (MGIT)

Round bottom tubes

Mycobacterial Growth Indicator Tube (MGIT)

- Positive signals are obtained in 10-12 days.
- MGIT can also be used as a rapid method for the detection of drug resistant strains of Mtb directly from acid-fast smear positive samples as well as from indirect drug susceptibility studies.

- Advantages over BACTEC
  - Cheaper.
  - No problem of radioactive waste disposal.

J Clin Microbiol 1999;37: 45-48

Detection and identification of mycobacteria directly from clinical samples

- **Genotypic Methods :**
  - PCR
  - LAMP
  - TMA / NAA
  - Ligase chain reaction
- **Phenotypic Methods :**
  - FAST Plaque TB

Polymerase Chain Reaction (PCR)

- Essentially PCR is a way to make millions of identical copies of a specific DNA sequence , which may be a gene, or a part of a gene, or simply a stretch of nucleotides with a known DNA sequence, the function of which may be unknown.

- A specimen that may contain the DNA sequence of interest is heated to denature double stranded DNA.

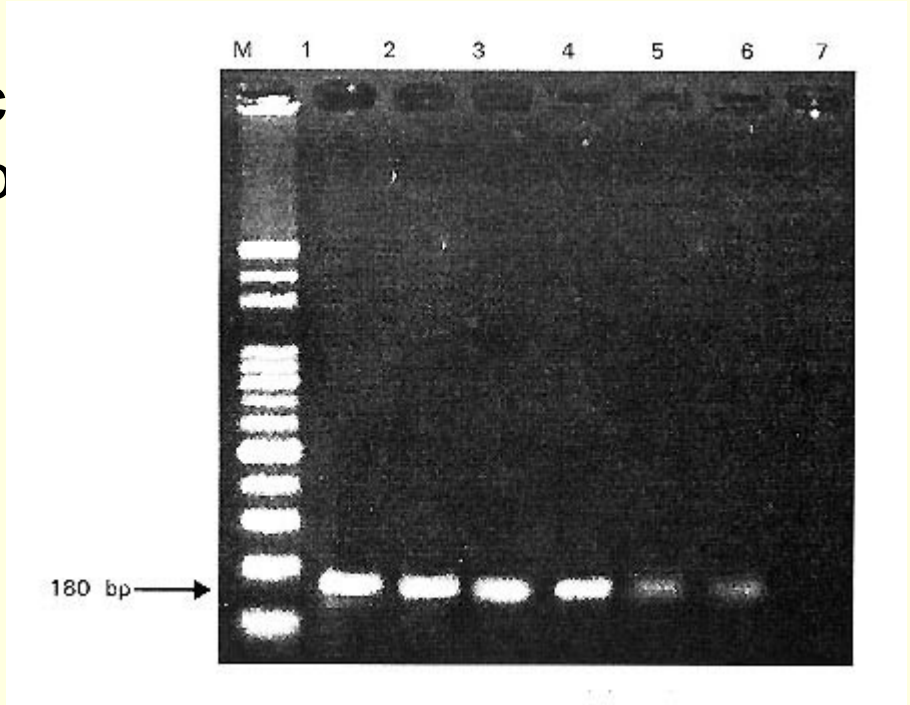
Polymerase Chain Reaction (PCR)

- Specific synthetic oligonucleotide primers bind to the unique DNA sequences of interest and a heat stable DNA polymerase (*Thermus aquaticus*) extends the primer to create a complete & complimentary strand of DNA.

- This process is repeated sequentially 25-40 times, thereby creating millions of copies of target sequence.

Polymerase Chain Reaction (PCR)

- The amplified sequenc
  can then be detected b
  agarose gel
  electrophoresis.

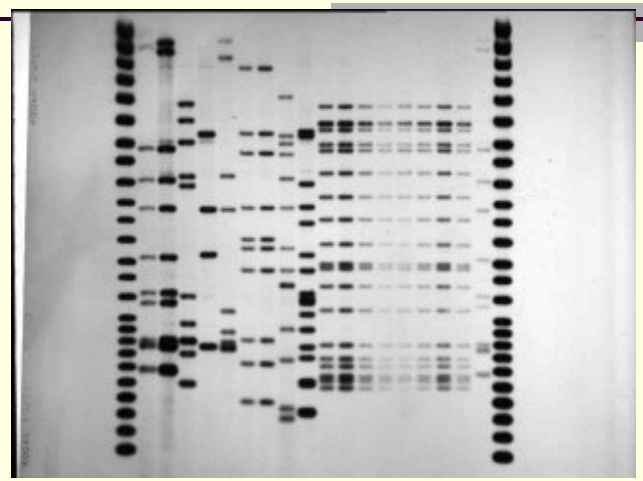


Polymerase Chain Reaction (PCR)

- 65 Kd antigen (HSPs):
  - Used earlier
  - Heat shock protein believed to be distinct from other bacterial HSPs.
  - This gene is identical in all species of mycobacteria.
  - Therefore unsuitable for detecting M.tb, particularly in areas where species like M.avium or M.kansasii are prevalent.

Polymerase Chain Reaction (PCR)

- IS6110 :
  - It is a transposon which are self replicating stretches of DNA.
  - Function not known.



- This sequence has been found in the M.tb complex organisms (M.tb, M.africanum, M.microti, M.bovis).
- IS6110 sequence generally occurs only once in M.bovis but is found as often as 20 times in certain strains of M.tb, thus offering multiple targets for amplification.

Polymerase Chain Reaction (PCR)

- With recent modification PCR can detect even a fraction of a bacilli.

- Role in pulmonary TB :
  - Detects nearly all smear +ve and culture +ve cases.
  - Useful technology for rapid diagnosis of smear –ve cases of active TB.
  - Able to identify 50-60% of smear -ve cases; this would reduce the need for more invasive approaches to smear -ve cases.

- Distinguish M.tb from NTM in smear +ve cases as IS6110 sequence is not found in NTM.*
- Should not be used to replace sputum microscopy.
- Sensitivity, specificity, & PPV for PCR is 83.5%, 99% & 94.2% respectively.**

Am Rev Respir Dis 1991; 144:1160

***** J Clin Microbiol 1999;31: 2049-2055***

Polymerase Chain Reaction (PCR)

- Role in Extrapulmonary TB
  - Limited Role
  - No comprehensive large series comparing the yield of PCR with other available approaches has been published.
  - But at present, it is valuable adjunct in the diagnosis of TBM, pleurisy, pericardial TB & other condition in which yield of other tests are low.

Polymerase Chain Reaction (PCR)

- Disadvantages :
  - Very high degree of quality control required.
  - Variation from lab to lab remain significant.
  - In pts. on ATT, PCR should not be used as an indicator of infectivity as this assay remains +ve for a greater time than do cultures.*

Am J Respir Crit Care Med 1997;155: 1804-1854

- High false +ve results in patients previously treated with ATT in contacts of sputum +ve active cases.
- High Cost.
- So, better understanding of how to use these tests in conjunction with available clinical information is essential.*

**Thorax 1992;47:690-694*

LAMP*

- Loop-mediated isothermal amplification.
- It is a novel nucleic acid amplification method in which reagents react under isothermal conditions with high specificity, efficiency, and rapidity.
- LAMP is used for detection of *M.tb* complex, *M.avium*, and *M.intracellulare* directly from sputum specimens as well as for detection of culture isolates grown in a liquid medium (MGIT) or on a solid medium (Ogawa's medium).

*Iwamoto T et al *J Clin Microbiol 2003;41 :2616-2619*

LAMP

- This method employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA.
- Species-specific primers were designed by targeting the *gyrB* gene.
- Simple procedure, starting with the mixing of all reagents in a single tube, followed by an isothermal reaction during which the reaction mixture is held at 63°C.
- 60-min incubation time.

LAMP

- Due to its easy operation without sophisticated equipment, it will be simple enough to use in:
  - Small-scale hospitals,
  - Primary care facilities
  - Clinical laboratories in developing countries.
- Difficulties :
  - Sample preparation
  - Nucleic acid extraction
  - Cross-contamination.

TMA / NAA

- Transcription Mediated Amplification (TMA).
- Nucleic Acid Amplification (NAA).
- These techniques use chemical rather than biological amplification to produce nucleic acid.
- Test results within few hours.
- Currently used only for respiratory specimens.

Ligase Chain Reaction

- It is a variant of PCR, in which a pair of oligonucleotides are made to bind to one of the DNA target strands, so that they are adjacent to each other.

- A second pair of oligonucleotides is designed to hybridize to the same regions on the complementary DNA.

Ligase Chain Reaction

- The action of DNA polymerase and ligase in the presence of nucleotides results in the gap between adjacent primers being filled with appropriate nucleotides and ligation of primers.

- It is mainly being used for respiratory samples, and has a high overall specificity and sensitivity for smear +ve and –ve specimens.

FAST Plaque TB

- It is an original phage based test.
- It uses the mycobacteriophage to detect the presence of M.tb directly from sputum specimens.
- It is a rapid, manual test, easy to perform and has a higher sensitivity than microscopy, in newly diagnosed smear +ve pts.*

*Int J Tuberc Lung Dis 1998;2: 160

FAST Plaque TB

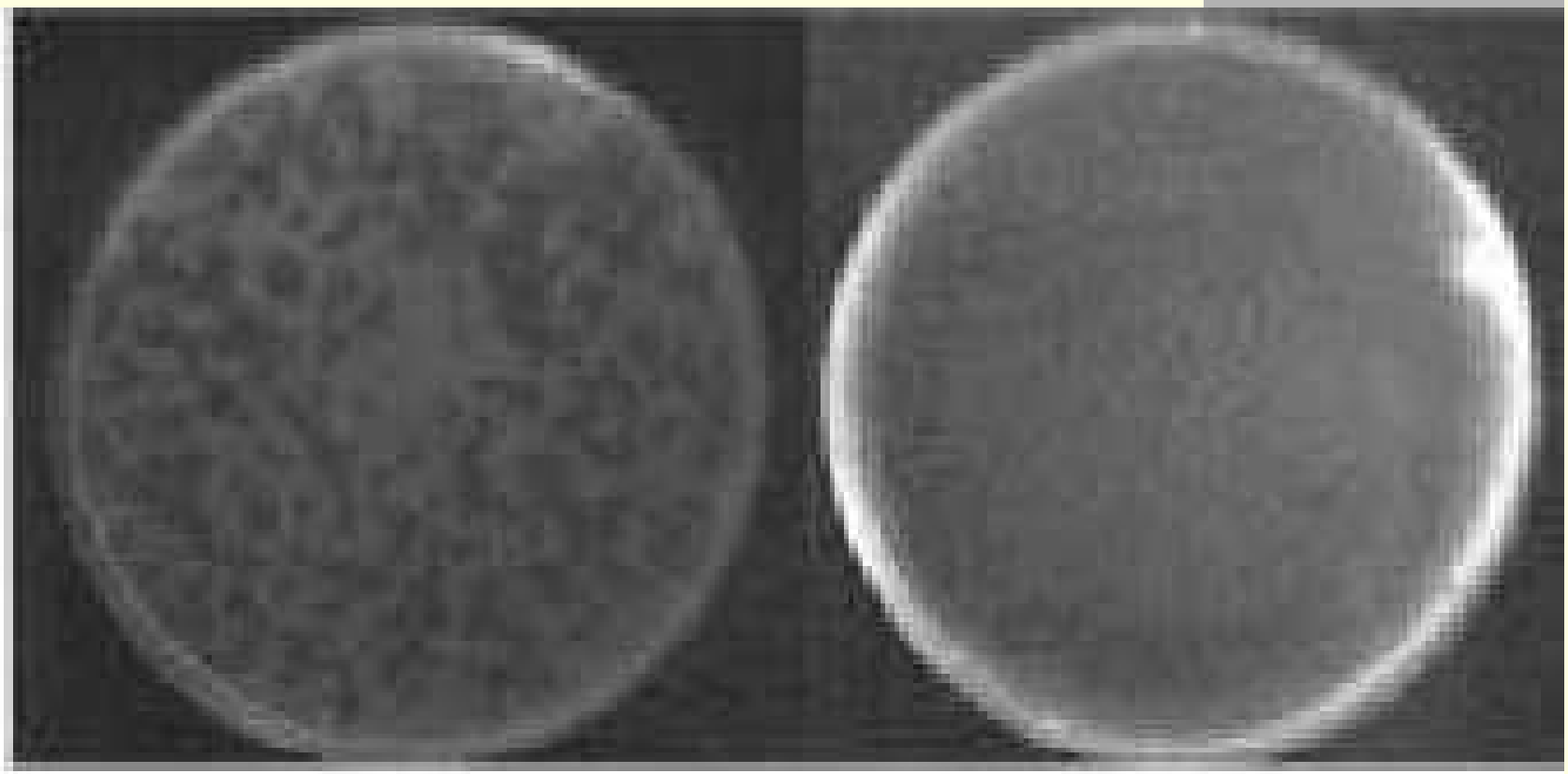


Figure 2 : Positive (>20 plaques) Negative (No plaques)

Indirect Methods

- **Antibody detection :**
  - TB STAT-PAK
  - ELISA
  - India test TB
- **Antigen detection :**
  - TB MPB 64 patch test.
  - Quantiferon-GOLD test.
- **Biochemical Assays :**(ADA, Bromide Partition, Gas Chromatography).

Antibody Detection

TB STAT-PAK

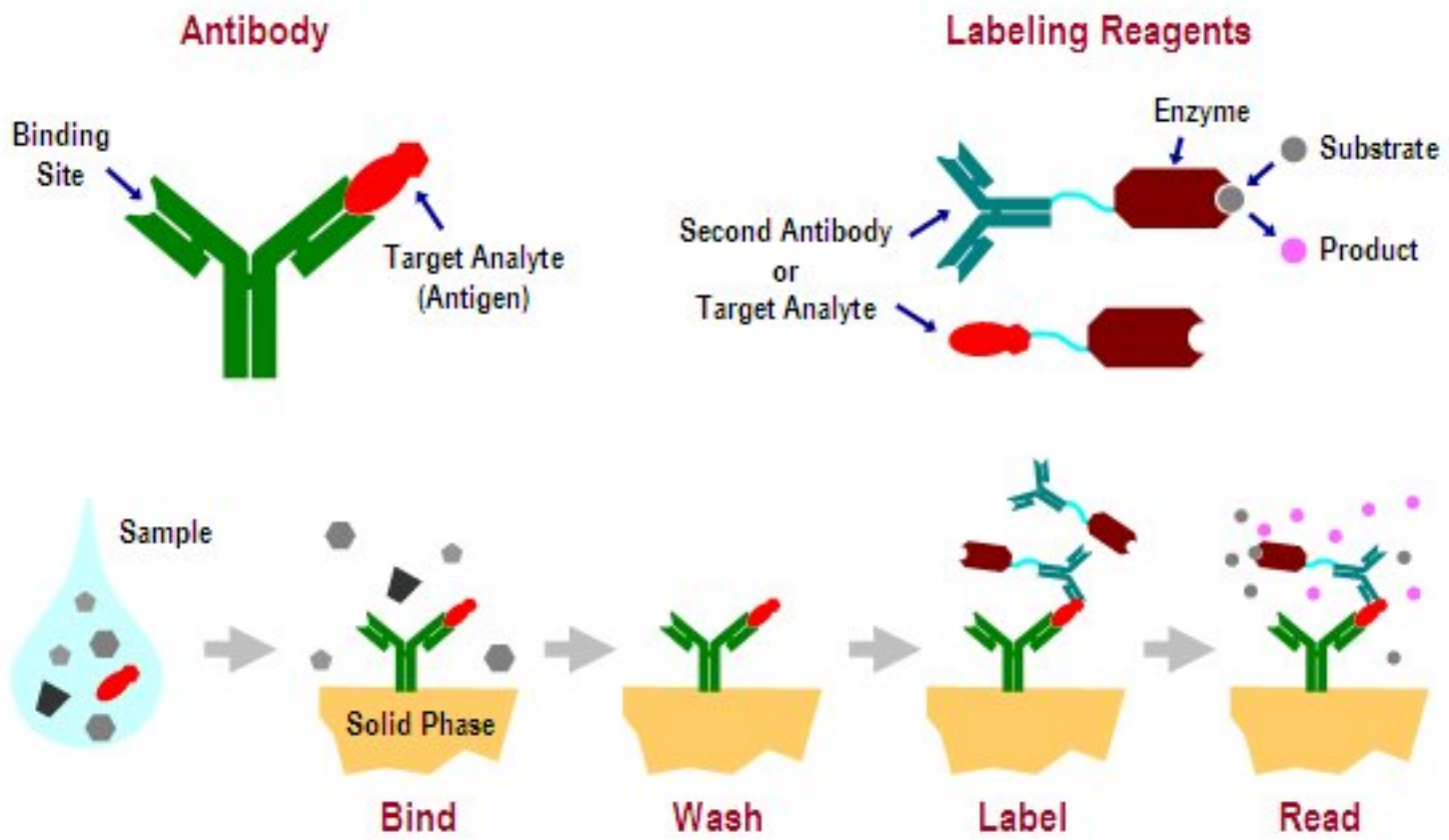
- Immuno-chromatographic test.
- Has been evolved with a capability to differentiate between active or dormant TB infection in whole blood, plasma or serum.
- Its value in in disease endemic countries is yet to be ascertained.*

Eur Resp J 1995;8: 676

Antibody detection by ELISA.

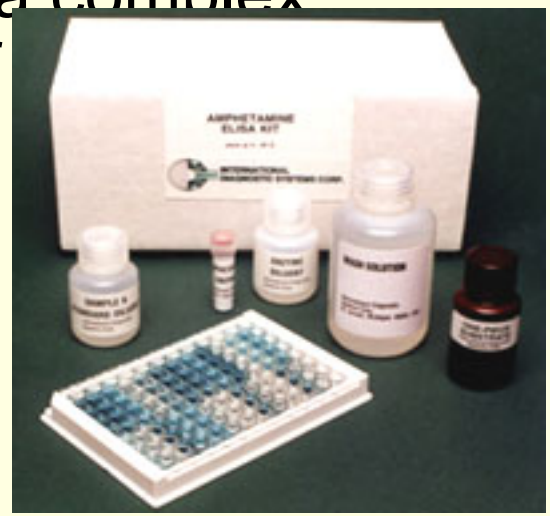
- Several serodiagnostic tests, principally those using ELISA methodology for measurement of IgG Ab are available.
- 38-Kd Ag provides serodiagnostic test with most favorable test characteristics described, but is limited by the lack of purified Ag.
- Serum IgG Ab are observed to rise during the first 3 months of therapy but fall after 12-16 months.

ELISA


Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read

Antibody detection by ELISA.

- **Other purified antigens to which antibodies are detected :**
  - 30 Kd protein antigen
  - 16 Kd heat-shock antigen
  - Lipoarabinomannan(LAM) – LAM is a complex glycolipid associated with cell wall of is produced in substantial quantities by growing M.tb.
  - A60 antigen
  - ES31/41 antigen



Antibody detection by ELISA.

- IgM Ab levels have usually been found to be so low that their reliable measurement has been difficult.
- Serodiagnosis with crude Ag gives high false positive results.
- These tests lack specificity because polyclonal Ab are used.
- Use of monoclonal antibodies have increased their specificity.

Antibody detection by ELISA.

- It takes several months after diagnosis for patients with pulmonary TB to reach maximum antibody titers so that serodiagnosis appears to be more useful in chronic extrapulmonary disease (bone or joint) than in acute forms (miliary, TBM).

- Serodiagnosis also has limited utility in smear negative patients with minimal PTB; In pediatric TB & in disease endemic countries with high infection rates.

Antibody detection by ELISA.

- ELISA also has limited diagnostic potential in AIDS prevalent population.*
- Tests are expensive, require trained personnel & difficulty in distinguishing Mtb & NTM.
- Serologic tests have not yet demonstrated sufficient performance to warrant routine use in control programs.

** Int J Tuberc Lung Dis 2000;4132: 5152-5388*

Antibody detection by ELISA.

- Sensitivity and specificity of ELISA serodiagnostic tests using measurement of serum IgG Ab to selected mycobacterial Ag:

Antigen	Sensitivity	Specificity
38 Kd	49-89	98-100
30 Kd	62-72	97-100
16 Kd	24-71	97-99
LAM	26-81	92-100
A60	71-100	71-95

Antibody detection by ELISA.

- The detection of mycobacterial antigens by immunoassay in clinical specimens with high & variable protein content is difficult.
- Detection in sputum presents even greater clinical problem because sputum is a non-homogenous gel .
- False positive rates are high.
- Abandonment of this diagnostic tool.

Insta test TB

- It is a rapid in vitro assay for the detection of antibody in active TB disease using whole blood or serum.

- The test employs an Ab binding protein conjugated to a colloidal gold particle and a unique combination of TB Ags immobilized on the membrane.*

Tuberc. Lung Dis 1998;2: 541

Antigen Detection

TB MPB 64 patch test

- MPB 64 is a specific mycobacterial antigen for M.tb complex.
- This test becomes +ve in 3-4 days after patch application and lasts for a week.
- Specificity~100%, Sensitivity~98.1%.*
- This promising test has been reported so far only in one setting in Philippines and needs to be carried out in other settings.

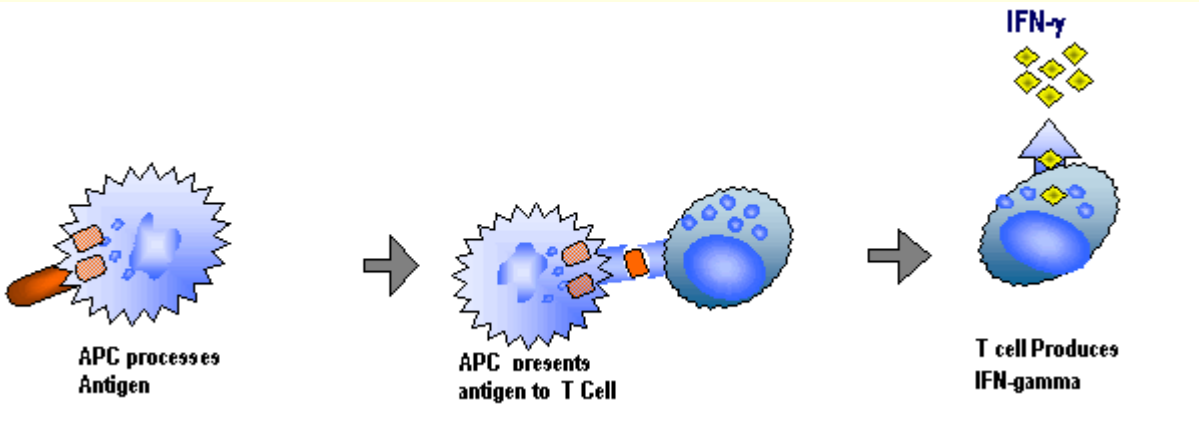
Ind J Tuberc Lung Dis 1998;2: 541

Quantiferon-GOLD

- Due to advances in molecular biology and genomics, an alternative has emerged for the first time in the form of a new class of *in vitro* assays that measure interferon (IFN-γ) released by sensitized T cells after stimulation by *M. tuberculosis* antigens.
- Measures immune reactivity to *M.tb.*

Quantiferon-GOLD

- Interferon-γ assays measure cell-mediated immunity by quantifying IFN-γ released from sensitized T cells in whole blood/PBMCs incubated with TB antigens.



- **QuantiFERON-TB ® test (Cellestis, Australia**

  – Commercially available.
  – Measures amount of IFN-γ produced. (*ELISA*)
  – FDA-approved for the detection of LTBI, 2001.

- **ELISPOT assay (Oxford, UK)**

  – Similar to QFT.
  – Measures number of reactive lymphocytes.
  – Not commercially available.

Quantiferon-GOLD

- Early assays employed PPD (same specificity problems as the TST).
- Newer assays (e.g., QFT-Gold) employ TB-specific antigens: ESAT-6 and CFP-10.
- Proteins encoded within the region of difference 1 of *M.tuberculosis.*
- Not shared with the BCG sub-strains and most NTM (except: *M. kansasii, M. szulgai, M. marinum and non-pathogenic M.bovis*).

Species Specificity of ESAT-6 and CFP-10

Tuberculosis complex	Antigens ESAT	CFP
M tuberculosis	+	+
M africanum	+	+
M bovis	+	+
BCG substrain		
gothenburg	-	-
moreau	-	-
tice	-	-
tokyo	-	-
danish	-	-
glaxo	-	-
montreal	-	-
pasteur	-	-

Environmental strains	Antigens ESAT	CFP
M abcessus	-	-
M avium	-	-
M branderi	-	-
M celatum	-	-
M chelonae	-	-
M fortuitum	-	-
M gordonii	-	-
M intracellulare	-	-
M kansasii	+	+
M malmoense	-	-
M marinum	+	+
M oenavense	-	-
M scrofulaceum	-	-
M smegmatis	-	-
M szulgai	+	+
M terrae	-	-
M vaccae	-	-
M xenopi	-	-

Quantiferon-GOLD

- Improved specificity: able to distinguish between TB and NTM, BCG infection.
- Studies in contacts, HIV infected and children underway.
- Recommended for use in “ALL circumstances in which the tuberculin skin test is currently used”.*
- Includes contact investigations, immigrant evaluation, surveillance (e.g. healthcare workers).

Mazurek et al MMWR 2005;54:15

IGRAs Vs. TST

- TST
  - *In vivo*
  - Single antigen
  - Boosting
  - 2 patient visits
  - Inter-reader variability
  - Results in 2-3 days
  - Read in 48-72 hrs
- IGRAs
  - *In vitro*
  - Multiple antigens
  - No boosting
  - 1 patient visit
  - Minimal inter-reader variability
  - Results in 1 day
  - Stimulate w/i 12 hrs

IGRAs Vs. TST

- QFT-g vs. TST Agreement = **83.6%***
- Factors associated with discordance :
  - Prior BCG
  - Non-tuberculous mycobcateria immune reactivity
  - Site bias in reading TST
  - TB Treatment

Mazurek et al JAMA 2001;286:1740

Biochemical markers of Diagnosis

- Adenosine deaminase. (ADA)
- Bromide partition test.
- Gas chromatography of mycobacterial fatty acids (Tuberculostearic acid).

Adenosine Deaminase (ADA)

- It is an enzyme of purine metabolism. The level of this enzyme is 10 times higher in lymphocytes (T cells >B cells) than in RBC.
- Whenever there is cell mediated immune response to an antigenic stimuli, the ADA levels are the highest.
- ADA is measured by the colorimetric method of Giusti.

- The enzymatic reaction is:

$$\text{Adenosine} + H_2O + \text{ADA} = \text{Inosine} + NH_3 + \text{ADA}$$

- The amount of ammonia liberated is measured by the colorimetric method.

	Cut-off	Sensitivity	Specificity
Pleural Fluid	50 IU/ml	95%	100%
Ascitic Fliud	32.3 IU/ml	89%	98%
CSF	9 IU/ml	100%	100%

Bromide Partition Test

- The partition of bromide ion between serum and CSF after a loading dose reflects the integrity of the blood brain barrier.
- Either by direct chemical measurement or by using an isotopic tracer, the ratio of bromide in serum to that in CSF can be estimated.
- Values <1.6 are characteristic of TBM.

- In different studies the sensitivity and specificity of this test has been found to be near 90%.*
- It may be false +ve in herpes simplex, listeria, mumps, measles, pyogenic meningitis and hypothyroidism.
- With the availability of better tests, this test has been given up.

*Taylor J et al. J Clin Microbiol 1999; 34: 56-59

Tuberculostearic Acid (TBSA)

- TBSA is found in the cell wall of mycobacterium.
- It is identified by gas chromatography or mass spectrophotometry.
- It is a costly investigation and requires complex analytical equipment. (Seldom used)
- Sensitivity >95%,Specificity>99%.*

* *French M et al. J Clin Microbiol 1998; 54: 987-990*

CT Scan and MRI Scan in the diagnosis of TB

- The advent of CT and MRI imaging in the last two decades has redefined the approach in analysis of various diseases including TB.*

- CT and MRI have shown several advantages over conventional radiology in early diagnosis and follow-up of TB in different parts of the body.

*Buxi TBS Indian J Pediatr 2002;69:965-972

Pulmonary TB :

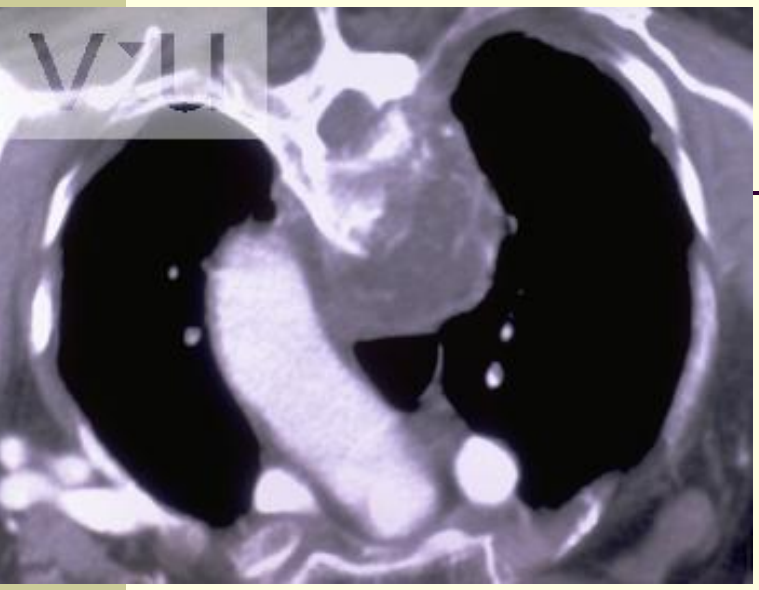
- Lobar Pneumonia
  - CT is superior than plain CXR in picking up the consolidation, atelectasis and the hilar LN thereby making the diagnosis easy.
  - MRI reveals some of these changes, however, CT is the diagnostic modality of choice in such cases.
- Bronchopneumonia
  - On CT it is usually B/L and widespread, not always symmetrical involvement of lungs.

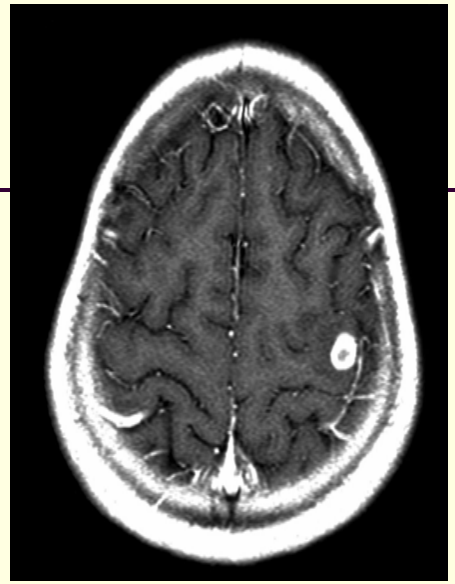
- Hilar and Mediastinal Lymphadenopathy

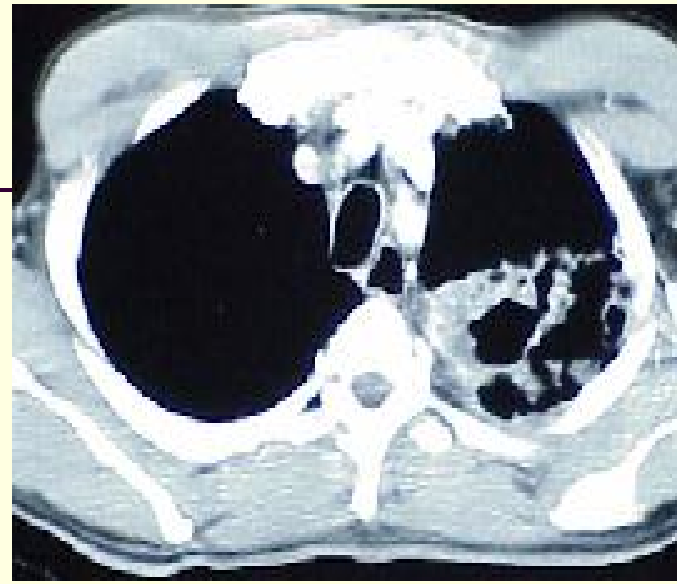
  - CT and MRI depict the hilar and mediastinal LN equally well.
  - Calcification in the nodes is however better seen on CT.
  - Necrosis is seen as focal areas of low attenuation on a CECT.
  - On MRI focal necrosis is seen as areas of increased signal intensity on T2W images.

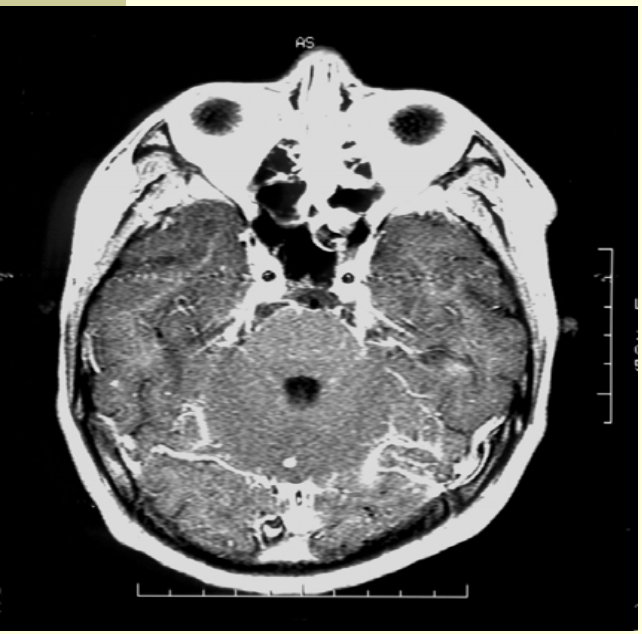
- EBTB

  - HRCT is sensitive in the detection of early endobronchial spread of disease.









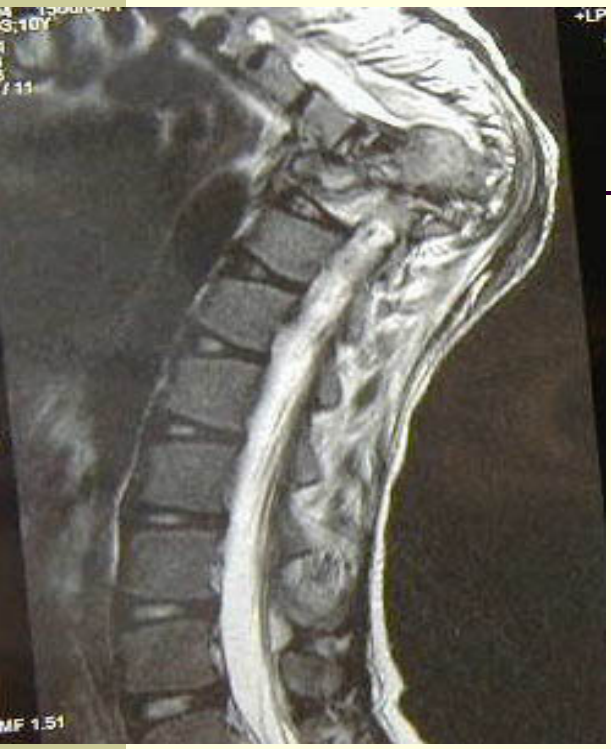
CT Images of TB in different parts of the body.

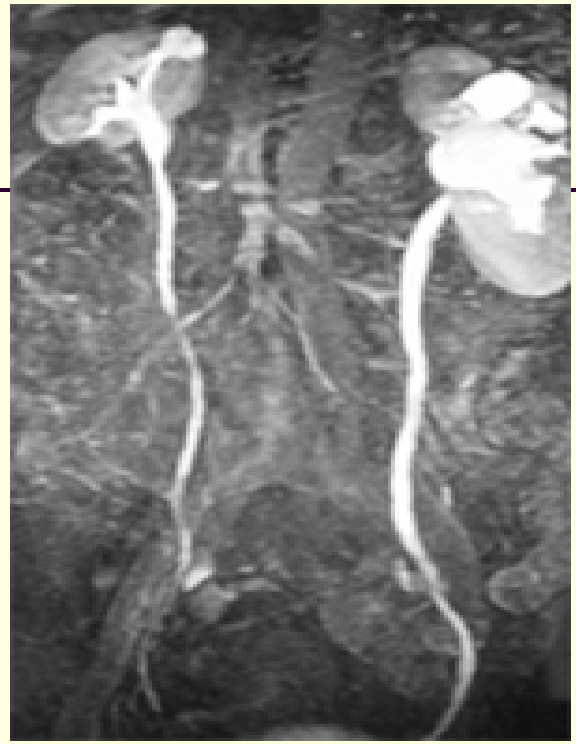
- Miliary TB
  - Earliest form of miliary TB is detectable on HRCT.
  - Coalescing nodules result into patchy irregular opacities and HRCT shows this variation effectively and has been described as “snowstorm appearance”.
  - HRCT shows cavitation, which is not evident on plain CXR.

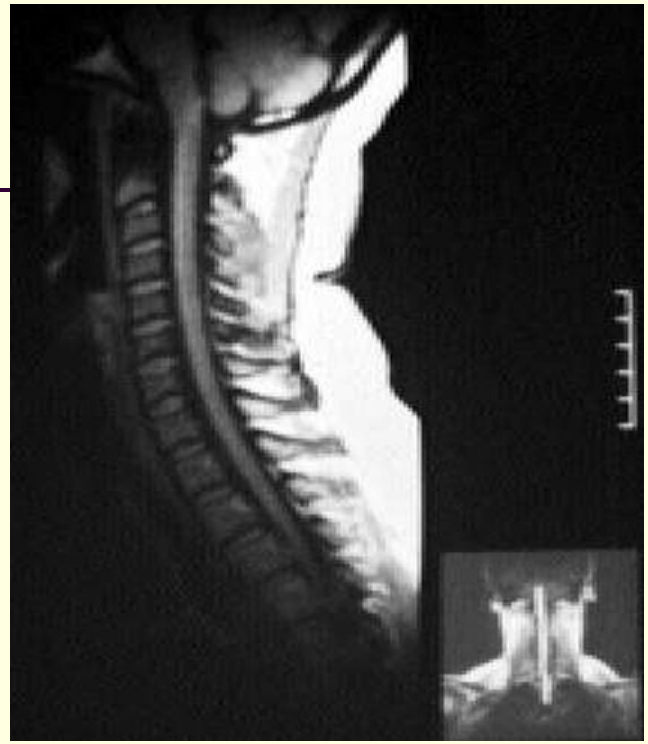
- Pleural Effusion
  - CT is sensitive to diagnose and define even minimal pleural effusion/pleural calcification.
  - Pleural fluid is seen on inversion recovery MR images as areas of increased signal intensity along the inner aspects of the chest wall.

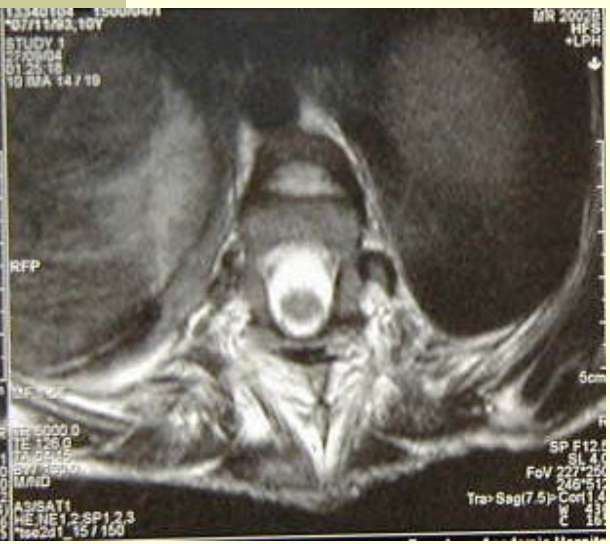
Skeletal TB

- Pott's Disease (vertebral TB)
  - CT and MRI helps in demonstrating a small focus of vertebral body involvement and defining the extent of the disease.
  - CT/MRI help to evaluate TB involving the cranio-vertebral junction, sacro-iliac joint and posterior appendages.
  - They are also helpful in assessment of spinal canal encroachment , posterior element involvement and in deciding the surgical approach.









MRI Images of TB in different parts of the body.

- **GIT TB**
  - Strictures of the small bowel, mucosal edema and thickening are well visualized on CT.
  - MRI depicts the para-aortic, aortocaval and mesentric lymph nodes effectively.
- **GUT TB**
  - Various patterns of hydronephrosis may be seen at MR urography.
  - MRI helps to differentiate macronodular TB lesions from the other mass lesions.

In the end…

- Today, although many new techniques are available for the diagnosis of TB and also for detection and identification of M.tb, direct microscopy is the only feasible method recommended for TB control program in India.

- Most of the new techniques described involve prohibitive expenditure, expertise & quality control, putting them out of reach of many labs in developing countries.

All the best..