SPECIMEN

PULMONARY

• SPUTUM
• LARYNGEAL SWABS
• BRONCHIAL WASHINGS
• GASTRIC LAVAGE (IN SMALL CHILDREN)

EXTRA-PULMONARY

• URINE
• CSF
• JOINT FLUID
• BIOPSY MATERIAL
• BLOOD
• OTHER BODY FLUIDS

TRANSPORTED AT 4°C IN CASE OF DELAY. IT PREVENTS MULTIPLICATION OF NON-MYCOBACTERIAL PATHOGENS
Decontamination & Concentration

- Petroff’s method – smear, culture, animal inoculation

- NALC combined with 2% NaOH – can be used for culture in automated systems

- Done in Class II biosafety cabinets
Diagnosis

- LED MICROSCOPY
- CONVENTIONAL CULTURE METHODS
  - SOLID MEDIA
  - LIQUID CULTURES
- NAT
- PHAGE BASED ASSAYS
- RAPID IDENTIFICATION OF MYCOBACTERIAL SPECIES
Acid fast staining - smear
<table>
<thead>
<tr>
<th>Result</th>
<th>Grading</th>
<th>No. of Fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10/field</td>
<td>+VE</td>
<td>3+</td>
</tr>
<tr>
<td>1-10/field</td>
<td>+VE</td>
<td>2+</td>
</tr>
<tr>
<td>10-99/100 field</td>
<td>+VE</td>
<td>1+</td>
</tr>
<tr>
<td>1-9/100 field</td>
<td>+VE</td>
<td>SCANTY</td>
</tr>
<tr>
<td>No. of AFB in 100 field</td>
<td>-VE</td>
<td>-</td>
</tr>
</tbody>
</table>

Intensity of infection

Infectedivity of the patient
Fluorochrome staining
**Culture Media**

**Conventional Culture** – Sensitivity as few as 10-100 Bacilli/ml

- Egg Based
  - Egg Medium
    - Dorset
      - Petragninini
  - Bactec
  - Liquid medium
    - Middle-Brook 7H9
    - Dubos’, Proskauer, Beck’s, Sula & Sautton’s

- Agar Based
  - Middle-Brook 7H
  - 10 Middle-Brook 7H 11

- Blood Based – Tarshis
- Serum Based – Loeffler
CULTURE

Conventional culture tech

Culture media

- solid
  - Egg based
    - L-J medium
    - Dorset
  - Agar based
    - Middle - brook 7H 10
    - Middle - brook 7H 11
- liquid
  - Middle - brook 7H9
GROWTH ON L-J MEDIUM
READING OF CULTURE IN LJ MEDIA

ZN microscopy

→

AFB seen  No AFB seen

→

Mycobacteria species  Reported as contamination

→

M.TB  NTM

PNB :  No growth  Growth
Niacin:  Positive  Negative
BACTEC 460TB

- First automated system for Mycobacteria testing.
- Detection — 4-8 days
- Susceptibility to first line drugs — 4 -12 days

Measurement of a radioactive CO₂ elaborated by the metabolism of the radiolabeled palmitic acid by the growing Mycobacteria
Non Radiometric Systems

USE FLUORESCENCE QUENCHING SYSTEM IN A LIQUID
10 DAYS
BD BACTEC™
9000MB
MYCOBACTERIAL DETECTION SYSTEM

MGIT TUBES
(MANUAL)
BD BACTEC™ MGIT™ 960 SIRE Susceptibility Testing for *M. tuberculosis*
Non Radiometric Systems

MB BACT (BMx) or BacT/ALERT

Uses a colorimetric CO₂ sensor in each bottle to detect growth
Nitrate reductase assay (Griess test)

- GROWTH DETECTED BY
  REDUCTION OF NITRATE TO NITRITE
- DETECTED BY COLORIMETRIC REAGENTS
  - RAPID: 8 - 10 DAYS
  - INEXPENSIVE
**Microscopic observation drug susceptibility (MODS) test**

- Liquid, rapid, identification of cord forming colonies
- For the detection of MDR-TB, sensitivity 95%, specificity 100%, accuracy 98%
- TAT - 9 days
- Contamination – 3.8%
- Single sample – sufficient

**Limitations** – Inverted microscope,
- attentiveness of microscopist
REAL TIME PCR

GeneXpert

Automated Sample Prep,
Amplification and Detection

<120 minutes
LINE PROBE ASSAY (LPA)-HAIN’S TEST

- Specific oligos of DR genes impregnated on nitro cellulose strip
- PCR amplicons allowed to hybridize with oligos
- Positive hybridizations reveal dark bands
- Rapid
- Highly specific
- Sensitivity 98% for Rif
- Not all mutations in DRDR can be detected
- Need to have phenotypic method
Non-conventional phenotypic assays: Phage based assays

**Principle**

- Genetically engineered mycobacteriophage
- Infects and expresses luciferase gene
- Luciferase converts luciferin using ATP
- Photons measured
Table 1. Comparison of turnaround time and sensitivity for laboratory diagnosis of *Mycobacterium tuberculosis*

<table>
<thead>
<tr>
<th>Respiratory specimens from patients with suspected TB</th>
<th>Turn-around time</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFE smear</td>
<td>2 hours</td>
<td>&lt; 50%</td>
</tr>
<tr>
<td>LJ culture</td>
<td>2-8 weeks</td>
<td></td>
</tr>
<tr>
<td>MGIT 96¢ culture</td>
<td>1-6 weeks</td>
<td></td>
</tr>
<tr>
<td>Mycobacterial culture identification and drug susceptibility testing</td>
<td>2-3 weeks</td>
<td></td>
</tr>
<tr>
<td>BDProbeTec™</td>
<td>4-5 hours</td>
<td>86-92%</td>
</tr>
<tr>
<td>GenProbe AMTD™</td>
<td>4-6 hours</td>
<td>85-95%</td>
</tr>
<tr>
<td>ROCHE COBAS™</td>
<td>5-6 hours</td>
<td>78-97%</td>
</tr>
<tr>
<td>In-house IS6110 PCR</td>
<td>18 hours</td>
<td>88-94%</td>
</tr>
<tr>
<td>rpoB PCR sequencing for RIF resistance</td>
<td>72 hours</td>
<td>92%</td>
</tr>
<tr>
<td>Conventional Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8-10 weeks</td>
<td></td>
</tr>
</tbody>
</table>
LAMP test

• Loop mediated isothermal amplification of DNA
• Is carried out at constant temperature & does not require a thermal cycler
• Detection of amplification can be determined by photometry
• SYBR green dye can be used to visualise the colour change
• Quantification of DNA can be done
Detection of Microbial product

1. TUBERCULOSTEARIC ACID:
   GAS CHROMATOGRAPHY
   SPECTROMETRY

2. ADENOSINE DEAMINASE
   ELEVATED LEVELS
   TUBERCULOUS PLEURAL EFFUSION
   (SENSITIVITY 98%, SPECIFICITY 96%)
<table>
<thead>
<tr>
<th></th>
<th>Reaction $\geq 5$ mm of Induration</th>
<th>Reaction $\geq 10$ mm of Induration</th>
<th>Reaction $\geq 15$ mm of Induration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive persons</td>
<td>Recent immigrants (within 5 yr) from high-prevalence countries</td>
<td>Persons with no risk factors for tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Recent contacts of tuberculosis case patients</td>
<td>Injection drug users</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrotic changes on chest radiograph consistent with prior tuberculosis</td>
<td>Residents and employees of high-risk congregate settings (prisons and jails, nursing homes, hospitals and other health care facilities, residential facilities for patients with AIDS, and homeless shelters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with organ transplants and other immunosuppressed patients (receiving equivalent of $\geq15$ mg/day of prednisone for at least 1 month)</td>
<td>Children less than 4 years of age, or infants, children, and adolescents exposed to adults at high risk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HIGH SERUM ADA

- Increase in number of T lymphocytes & macrophages in pleural fluid
- Recirculation of activated T-cells
- TB clearance capacity of lungs is decreased
- ADA activity is greater in lymphocytes
- Activation of CMI leads to secretion of ADA
Nucleic acid Technology

- PCR BASED DNA AMPLIFICATION
- AMPLIFICATION TARGETING RIBOSOMAL RNA
- RFLP
- DNA FINGER PRINTING
Mantoux test

>10 mm induration at the injection site = positive

< OR 5mm – negative

6-9mm – equivocal

1TU PPD used – extreme hypersensitivity

Doses of 10 or 100 when 5TU negative
Mantoux test - Uses

- FOR DIAGNOSING ACTIVE INFECTION IN INFANTS & YOUNG CHILDREN
- TO MEASURE PREVALENCE OF INFECTION IN AN AREA
- TO SELECT SUSCEPTIBLE CASES
- AS AN INDICATION OF SUCCESSFUL VACCINATION
Quantiferon TB Gold

- Simple blood test
- Quantiferon is an interferon gamma (IFNγ) release assay (IGRA)
- Alternative to Mantoux test
- Requires only one patient visit
- Unaffected by BCG vaccination
- Blood of infected patient is stimulated with M. Tuberculosis specific Ag- T cells secrete IFNγ and its concentration is determined using ELISA
Thank You