SKELETAL TUBERCULOSIS-LAB DIAGNOSIS

Dr. ARCHANA G.J
Assistant Professor of Microbiology
Skeletal Tuberculosis

- 10-35% of cases of extra pulmonary TB.
- Ideally the diagnosis of TB infection should be confirmed by demonstration of tubercle bacilli in skeletal TB
- Load of bacteria in pulmonary lesion $10^7$-$10^9$ whereas in osteoarticular disease $<10^5$ – paucibacillary osteoarticular tissue
The causative agent is *Mycobacterium tuberculosis*.

Incidence of AFB in Osteoarticular TB (40-88%)
SPECIMEN

- Best material - centrifuged residue from large quantity of abscess. Cytocentrifugation enhances the sensitivity.

- Joint aspirate, pus.
- Aspirate of lymph node, skin and bone
- Bone marrow biopsy
- Curettings from walls of cold abscess,
- Curettings from lining of sinus tract.

- TRANSPORT-24 hrs
SAFETY CONSIDERATIONS

A Bio Safety Cabinet 2 (BSC 2)

- DEDICATED LAB FOR SPECIMEN PROCESSING
Microscopy

Acid fast bacilli - ZIEHL NEELSON STAINING

Fluorescent method for acid fast bacteria
LED Fluorescent Microscope—most sensitive.
Direct smear examination of tuberculous material obtained during operation/aspiration of joints/ bursae, tendon sheath or LN/ cavity may yield positive result if sample contains $>10,000$ bacilli /ml of specimen.

$10^4$-$10^6$ org/ ml are required for detection
CULTURE

- Culture
- Slow and insensitive
- Paucibacillary osteoarticular disease
- Egg based-LJ medium-2-6 weeks
- Agar based Middlebrook media 7H10,7H11- 3-4 Weeks.
- Liquid media Middlebrook media 7H12,7H13-2 weeks.
Radiometric Bactec 460 TB
MGIT 960 Mycobacteria detection system.
Versa Trek
MB / Bact system.
Septi check
Microcolony detection on solid media (rapid slide culture tech.)
Microscopic observation of broth cultures (MODS)
BACTEK RAPID RADIOMETRIC CULTURE SYSTEMS

- Radiometric Bactec 460 TB
  - Middlebrook media 7H12, or 7H13
  - Radio active index of Co$_2$ released from C 14 labelled palmitic acid measured
  - Growth index $\geq 10$ --- positive

- DETECTS GROWTH IN 9-14 days
Bactec 460 TB
Non radiometric methods

- **MGIT (Mycobacterial growth indicator tube)**
  - 7H12 broth.
  - Detects growth by monitoring $O_2$ consumption by means of fluorescent sensor
  - $O_2$ quenching is detected.

- Florescence detected by U.V. light

- Rapid, accurate & cost effective.
- Detection and drug screening
BACTEC MGIT 960

- Automated, continuous monitoring
- for every 60 minutes
The MPB-64 antigen detection by lateral flow assay

Differentiate MTB from MOTT
MODS-Microscopic Observation And Drug Susceptibility Assay
MODS (Microscopic observation of broth cultures)

Growth observed by inverted microscope in broth
Rapid Identification of Mycobacterial Isolates

- **ANALYSIS OF LIPID PROFILES-HPLC.**

- **DNA probes** - when used along with MGIT-identity -1-2 days.

- **Ribosomal r RNA based PROBES** - MORE SENSITIVE - LOWEST DETECTION LIMIT OF 100 ORGANISMS.

- **GENE AMPLIFICATION METHODS** - PCR -RFLP-sensitivity 1-10 copies-direct application on specimens is possible.
- Rapid microscopic evaluation of AFB.
- Culture
- DST
- NAAT/PCR of collected sample
Other amplification methods

- PCR
- Transcription mediated amplification (TMA)
- Ligase chain reaction (LCR)
- Strand displacement amplification (SDA)
- Nucleic acid sequence based amplification (NASBA)
- Branched DNA (b-DNA)
- Line probe assay (LiPA)
- Q Beta replicase
## Targets for Different Amplification Procedures

<table>
<thead>
<tr>
<th>Method</th>
<th>Target</th>
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</thead>
<tbody>
<tr>
<td>PCR</td>
<td>IS 6110, 65 KDa</td>
</tr>
<tr>
<td>TMA</td>
<td>16 s rRNA</td>
</tr>
<tr>
<td>SDA</td>
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<tr>
<td>bDNA</td>
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CB NAAT (Xpert MTB/RIF)

- Cartridge Based Nucleic Acid Amplification Test

- FIND & Cepheid (FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS)

- Fully automated

- Purifies, concentrates, amplifies (Real time PCR)

- Case detection & Rifampicin resistance testing

- Result – within 2 hours.

- December 2010 WHO endorsed it for use in endemic countries
Gene Xpert-CB NAAT (Xpert MTB/RIF)
- 3 specific primers & 5 molecular probes.

- Nested Real Time PCR for detection of *Mycobacterium tuberculosis* complex DNA and Rifampin resistance associated mutations of the *rpo B* GENE

- 5 genome copies/purified DNA.

These assays are useful in early confirmation of diagnosis in paucibacillary extra pulmonary forms-skeletal tuberculosis.

The GeneXpert TEST SHOWED A SENSITIVITY OF 95.6% AND SPECIFICITY of 96.2% FOR SPINAL TB.

RESULTS-4HRS. Compared to median of 35 days for cultures.

MDR TB were accurately diagnosed with the Gene Xpert test.
LAMP

- LOOP MEDIATED ISOTHERMAL AMPLIFICATION.
- 4 pairs of primers.
- 65 degree for 40 min.
Procedure

1. Prepare master mix.
2. Add DNA/RNA samples
3. LAMP amplification
4. Detection
   - Visual detection (by fluorescence)
   - Real-time turbidity detection

DNA/RNA extraction from samples

About 30 min

About 60 min
Detection methods

Visual methods

Real time

Optigene instrument for LAMP
Interferon Gamma Release Assay

- Quantiferon TB Gold
- T spot-TB test
- To distinguish latent tuberculosis infection from BCG vaccination.
ANTIGEN DETECTION

- Immuno assay system
- Excretory Secretory ES-31 MYCOBACTERIAL ANTIGEN -immuno diagnosis.
- Ig G ESTIMATION.
- The MPB-64 antigen detection by lateral flow assay
Diagnosis of MULTI drug-resistant tuberculosis (MDR-TB)

- INNO-LiPA Rif assay.
- *GenoType MTBDR* assays for rapid detection of rifampicin resistance.

- The INNO-LiPA Rif assay.
- *GenoType MTBDR* assays for the detection of rifampicin resistance in culture isolates.

- These have excellent sensitivity and specificity for rifampicin resistance, even when directly used on clinical specimens.
GenoType MTBDR plus
Thank You