Introduction and orientation of Xpert MTB/RIF Assay, how to order test

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Presentation Plan

• Brief introduction to CBNAAT
• Available Tests for TB Diagnosis
• Current Recommendations of RNTCP for use of these tests
• CBNAAT problems faced: solutions
• How to order CBNAAT testing
• Final comments
CBNAAT: An Introduction

Lab at KGMU

Tuberculosis Diagnostic Automated DNA Test

• Fully automated
• Detects MTB plus Rif resistance
• TAT 3 hours
• Minimal bio safety requirements and training
• Non conventional lab requirement
• Simplified mix of 6 complex technologies
Gene-Xpert: Automated Nucleic Acid Amplification Test

Xpert MTB/RIF

5, 20, 80 Samples per shift 500-1000

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Cartridge Design and Operating Principle

- Syringe Barrel
- RT-PCR Tube
- Rotary Valve
- Sonicator Dome
DNA molecules are mixed with dry PCR reagents.
Sample is automatically filtered & washed.
Ultrasonic lysis of filter-captured organisms to release DNA.
Nested real-time amplification & detection with internal process control.

Time to result: 1 h 45 min

GeneXpert

Concentrates bacilli & removes inhibitors
Sputum liquefaction & inactivation with 2:1 SR
Transfer of 2 ml after 15 min
End of hands-on work

Xpert MTB/Rif
Available Tests for TB diagnosis
TB - Diagnostic Test Coverage: visible shift in last decade

Symptoms  X-ray  Smear  Culture Solid  Culture Liquid  DST  Molecular Probes

Visible shift

Developed Countries

Developing Countries

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Tools for diagnosing TB: limited choice

- **ESTABLISHING DIAGNOSIS (TAT) (Sensitivity)**
  - AFB* demonstration *(2hrs) (1000 bacilli/ ml)*
    - ZN/ AR staining
  - Culture* *(2days-8 weeks) (100 bacilli/ ml)*
    - Liquid/ solid
  - NAAT/ PCR *(2days) (10 bacilli/ ml)*
    - Conventional/ real time (in-house/ commercial)
  - Gene -Xpert *(4 hrs) (10 bacilli/ ml) (detects R to rif)*

- **MTB/ MOTT*: once you have found one of the above test positive!
Current Recommendations of RNTCP for use of these tests

EVERYDAY ISSUES
Case classification for testing purpose

- Pulmonary
  - New (grp1)
    - AFB positive/ AFB negative
  - Treated in past (Grp2)
    - AFB positive/ AFB negative

- Extra-pulmonary (includes Pediatric TB and TB with HIV): Both New and old (Grp3)
  - AFB positive/ AFB negative

1. Case of New Pulmonary TB: Not treated in past

- Only test for MTB
- Do not look for Drug resistance (~3% MDR)
- Recommended test:
  - Only Microscopy: twice
  - One Morning sample, one spot sample
- If Positive treat: Treatment is Cat I
- If Negative: Use your clinical acumen act accordingly

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AFB Examination

- Methods available: ZN/ AR
- Fluorescent method is 10% more sensitive than ZN
- TAT: 2 hours
- Inexpensive; Reagent cost is ~2-5 Rs
- Available free at DMC, Dept. of Pulmonary Medicine
- Please use the facility
Microscopy (LED FM) facility at KGMU
2. A case of Pulmonary TB treated in past

- MDR suspect
- Detect M TB
- **AND** Drug resistance (~25% MDR TB)
- Past Treatment should be for > 4 weeks
- Look for records or else take detailed history
DR -TB Detection: Methods available

- CBNAAT
- Line Probe Assay
- Liquid Culture
- Solid Culture
Recommended Algorithm: Sputum

- AFB POS
  - LPA: if dispute in interpretation then
  - Liquid Culture

- AFB NEG
  - CBNAAT
LPA Facility
Culture & DST Facility (BSL-3 Lab)
3. Extra Pulmonary TB, Pediatric TB, TB- HIV: both new and treated cases

• Both AFB positive and Negative: **CBNAAT**
• Detects M. Tb and Rif Res
• Time taken: 4 hours
CBNAAT: problems faced
WHAT TO DO?
1. AFB Smear Positive and CBNAAT negative ("MTB not detected")?

• Answer:
• Do not repeat CBNAAT.
• Use LPA/ Liquid Culture
  – A fresh sample is required by lab
  – If not available left over of previous sample can be used if available
  – We examine sample for AFB before further processing
  – Because sample to sample variation in AFB positivity exists
  – If this sample is AFB positive: LPA
  – If AFB negative: Liquid Culture
AFB Smear Positive and CBNAAT negative (“MTB not detected”)? Cont...

- May be an indicator of MOTT/ NTM
- 5-7% of Mycobacterial isolates are MOTT
- Confirmed by culture only; takes time
- LPA can not confirm; only suggests
2. Samples from more than one site is submitted for examination

• ONLY ONE SAMPLE PER PATIENT IS TESTED
• Please DECIDE and MENTION on requisition form: the site of active disease
• If You will not decide which sample to test lab will decide: whose pt is this???
• If this sample is negative only then other sample MAY be tried
3. CBNAAT reports Rif sensitive but no sputum conversion with FLD

- No genotypic test is 100% accurate....
- Get a culture DST done: Phenotypic reporting is more accurate for M. TB
- DO NOT ASK FOR REPEAT CBNAAT Testing
- Sensitivity and specificity of CBNAAT is ~95%
- Enough literature to support this data

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4. Sample for Pediatric Pulmonary TB

- Gastric Aspirate: Morning empty stomach single sample
- Transport immediately (too acidic)
- Sample collected on full stomach is rejected
- Older children: Who can expectorate: sputum: treated as pulm TB
5. Sample for EPTB

- Sample from representative site eg: CSF, body fluid, pus etc are only acceptable sample
- REJECTED/ unacceptable SAMPLES:
  - Blood and serum
  - FNAC: in case there is no visible aspirate
  - Needle washings
  - Urine because contaminants are common
- POOR YIELD: may be rejected at times
  - Pus, tissue, blood containing samples are thick and have inhibitory proteins, take longer to process/ rejected
  - Pleural fluids are inflammatory fluid
Sample for EPTB cont..

• Quantity: As much as possible
• Quality: Blood contamination as minimum as possible, in screw capped sterile container, do not let the sample dry, No additives
• Transport: ASAP
6. Sample for Pulmonary Tb

- Sputum: not saliva
- Older and sick people can be asked to induce sputum sample: Steam inhalation
- Broncho alveolar lavage
- Food, paan, tobacco and supari contamination in sputum; common cause for sample rejection
- Quantity: 2-10 ml
- Quality: Mucoid avoid saliva
GIGO

- Expensive test
- Poor sample: wastage of resources
- Spend some time to ensure retrieving good quality sample

REPEATING TEST IS NOT POSSIBLE
7. Follow up of Pts on cat IV Treatment

• Response to follow up not tested by CBNAAT
• LC done for crucial months of follow ups: 1st and last 6 months of follow up (at 3, 4, 5, 6, 18, 21, 24 months)
• Solid culture done for 7, 9, 12 and 15 months of follow up
How to order test
DOS AND DON’TS
CBNAAT: when?

- Sputum from old cases (treated in past) if Sm –ve
- All EPTB with few exceptions
- Ped TB: all Gastric aspirates only
- TB HIV: all sample
CBNAAT: when not?

- Patients already classified and on treatment
- Lab confirmed MDR patients
- AFB positive Pulmonary TB patients
- Already once tested by CBNAAT
Sample with Annexure 1 form

- No Samples will be acceptable without annexure 1
- No samples can be entertained directly from outside KGMU
- Anybody needing service please contact DTO
- Anybody needing research project please approach through OR
If EP-TB case, please mention:
Type of sample
New or Retreatment case
HIV Status

If SL-DST is desired please mention:
(For SL DST)
Where to get annexure 1 form

- DOTS center/ DMC
- Pulmonary medicine Department in KGMU
- Every clinical department has a nodal officer
- If not please depute one
- CONTACT Prof Suryakant STF Chairman
Final note
Notification

- TB is a notifiable disease
- It is a legal duty of all medical practitioners to notify every TB patient
Detection of TB and DR TB: Issues with endemic countries

• Ideally EVERY TB suspect/patient needs test for drug resistance: should be available in a cost effective manner

   BUT

• In Endemic Country like India with enormous TB load and limited lab facility, management of TB is/should be different from other countries e.g. latent disease is not diagnosed and treated
Detection of TB and DR TB: Issues with endemic countries

• Till lab facilities can be expanded country wide...
  – New cases (Pulm TB): Establish the diagnosis
  – Treated in past (Pulm TB) (History taking/ documents): look for resistance??

With CBNAAT Rif resistance is tested on all EPTB, Pediatric TB, HIV- TB, Smear negative pulmonary TB
Current Role of Laboratory in TB management

• Detection of disease
  – Active/ Latent disease, Either treat or not

• Detection of drug resistance
  – Choice of treatment, Response to therapy (prognosis?)

• Assessing Response to Treatment
  – Smear or culture conversion

• Epidemiological studies
  – Source of infection, Strain relatedness etc..

• Research
Purpose of testing

Type of case

Diagnoses options available at TB Lab (IRL), KGMU

Sample

To monitor treatment response

Diagnosis

New pulmonary TB case

New TB case

MDR-TB suspect (Pulmonary)

MDR-TB case

Microscopy (LED FM)

Microscopy (LED FM)

XDR-TB suspect

New pulmonary TB case

EP-TB, Pediatric-TB, TB-HIV cases

MDR-TB suspect (Pulmonary)

XDR-TB suspect

New TB case

MDR-TB case

Microscopy (LED FM)

CBNAAT

First Line Liquid Culture -DST

(If, RIF resistance is in-determined in CBNAAT/LPA)

Baseline second line liquid culture -DST

(If, RIF resistance detected by any above test)

Solid Culture FL-DST

(If, LC-DST service of Lab get interrupted).

Second line liquid culture -DST

(for Ofloxacin & Kanamycin)

CBNAAT (for smear negative case)

LPA (for smear positive case)

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(If, RIF resistance is in-determined in CBNAAT/LPA)

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(If, LC-DST service of Lab get interrupted).

Second line liquid culture -DST

(for Ofloxacin & Kanamycin)
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Thank You