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

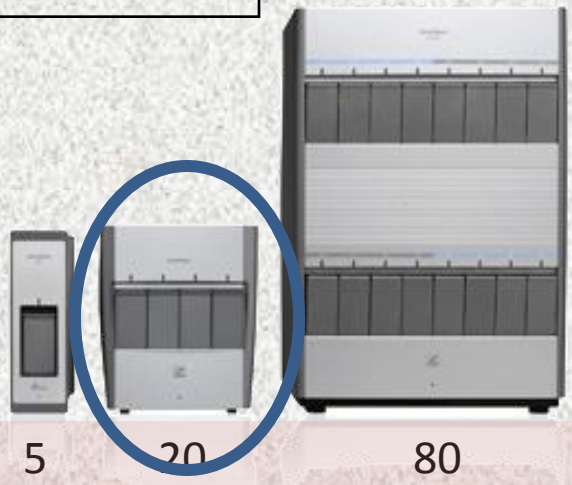
Lab at KGMU

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

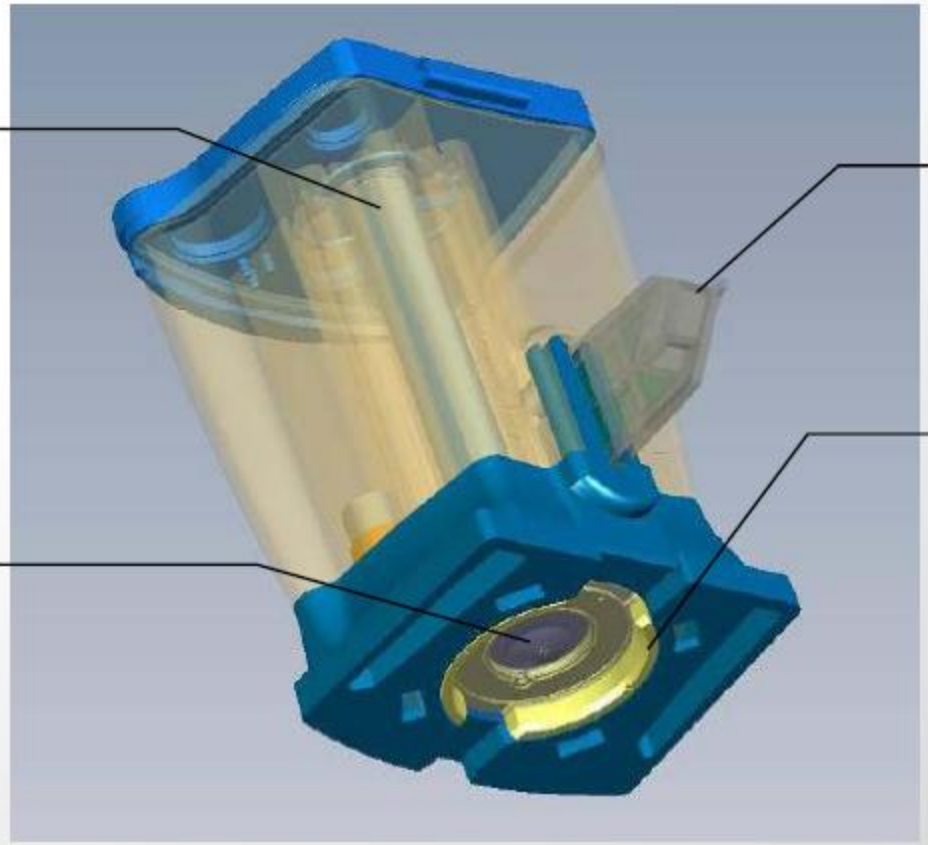
Gene-Xpert: Automated Nucleic Acid Amplification Test

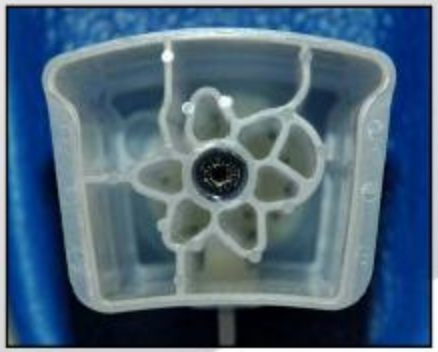
Xpert MTB/RIF



5	20	80	Samples per shift	500-1000

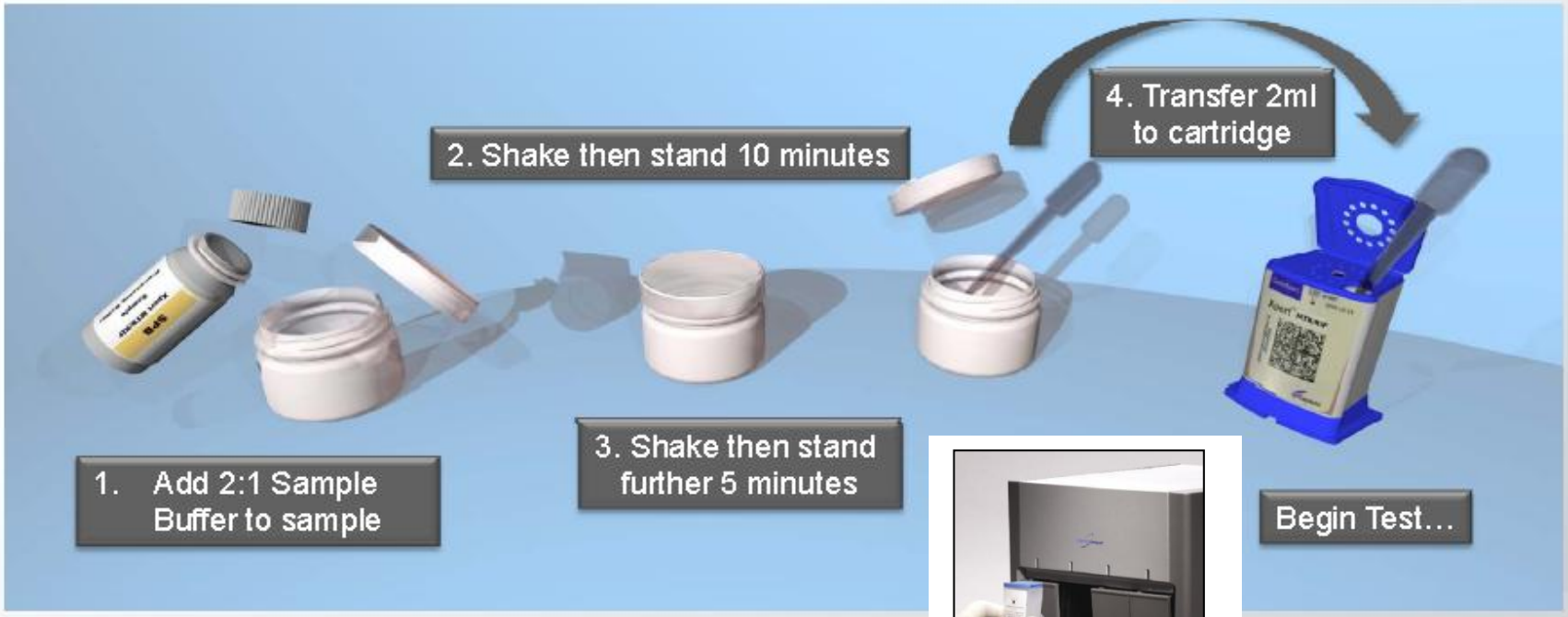
Cartridge Design and Operating Principle





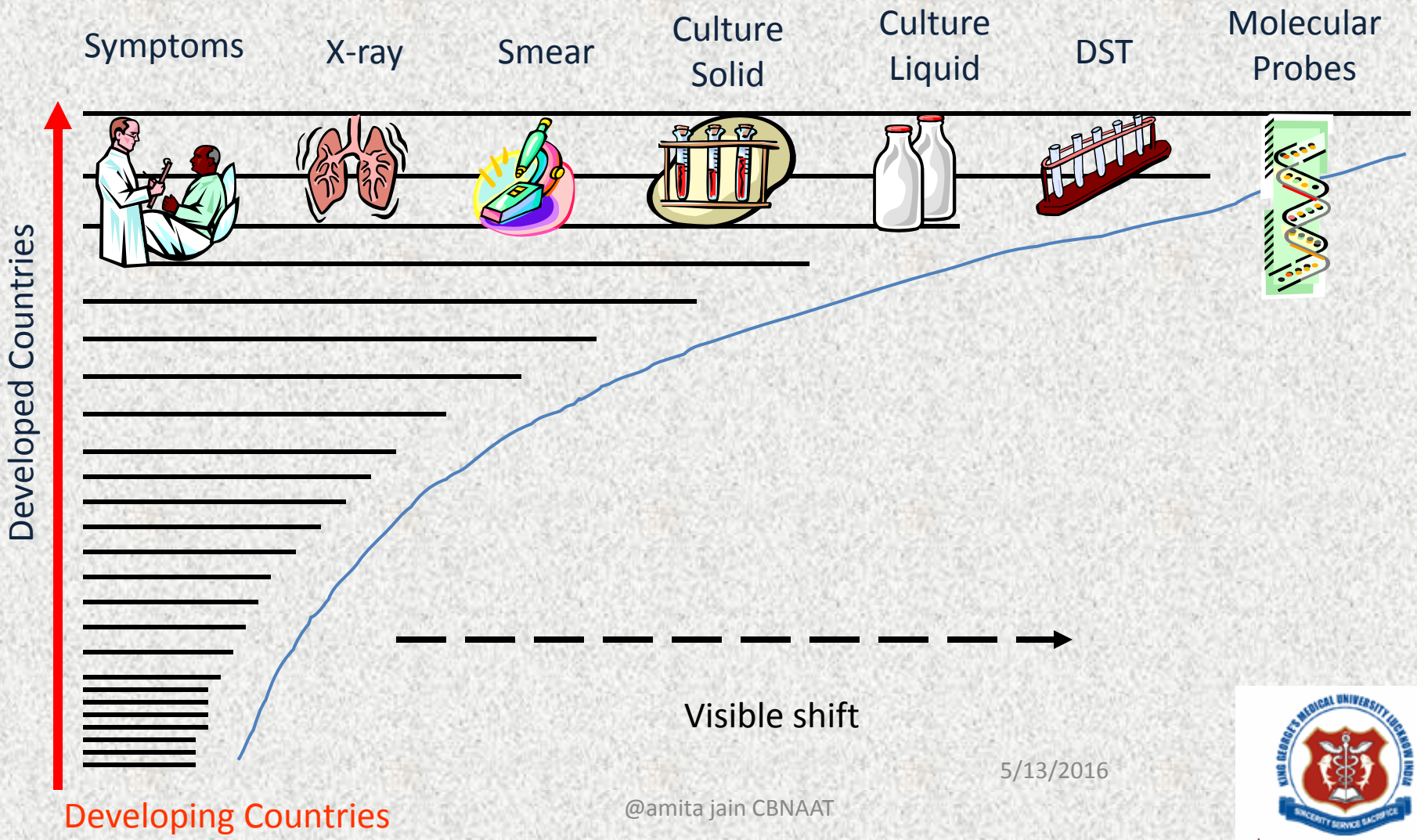
Xpert MTB/Rif

Simple Sample Processing – Direct Sputum



Available Tests for TB diagnosis

TB- Diagnostic Test Coverage: visible shift in last decade



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

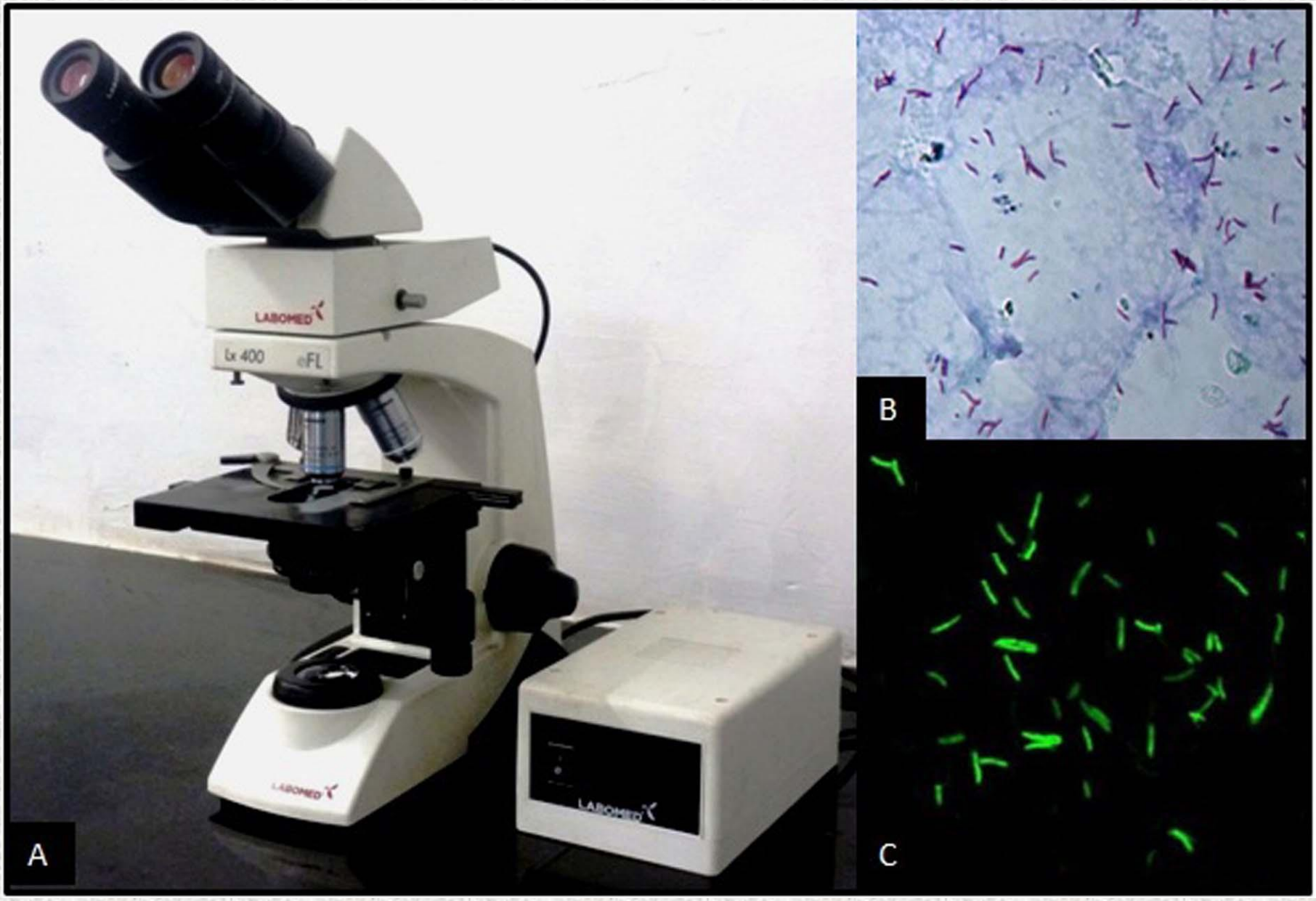
- Pulmonary
  - New (grp1)
    - AFB positive/ AFB negative
  - Treated in past (Grp2)
    - AFB positive/ AFB negative
- Extra-pulmonary (includes Pediatric TB and TB with HIV): Both New and old (Grp3)
    - AFB positive/ AFB negative

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

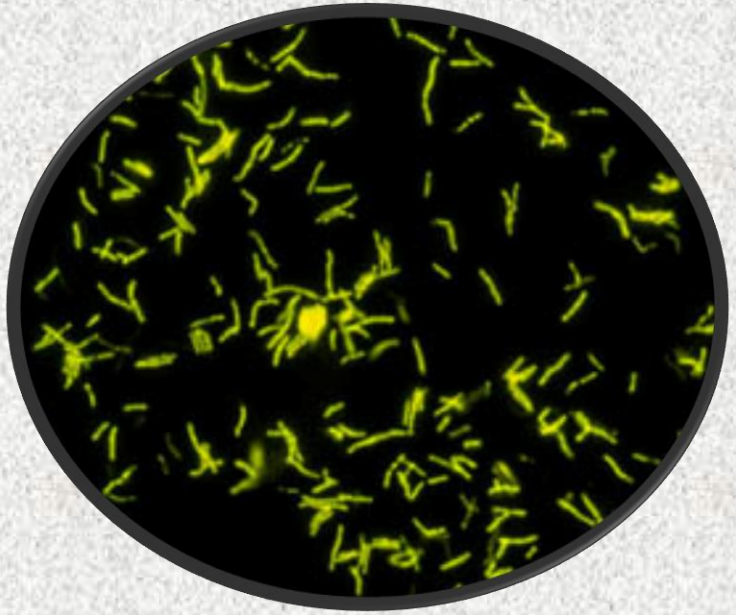
- Only test for MTB
- Do not look for Drug resistance (~3% MDR)
- Recommended test:
  - Only Microscopy: twice
  - One Morning sample, one spot sample
- If Positive treat: Treatment is Cat I
- If Negative: Use your clinical acumen act accordingly

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


LABOMED
Lx 400
FL
A
B
C

Microscopy (LED FM) facility at KGMU



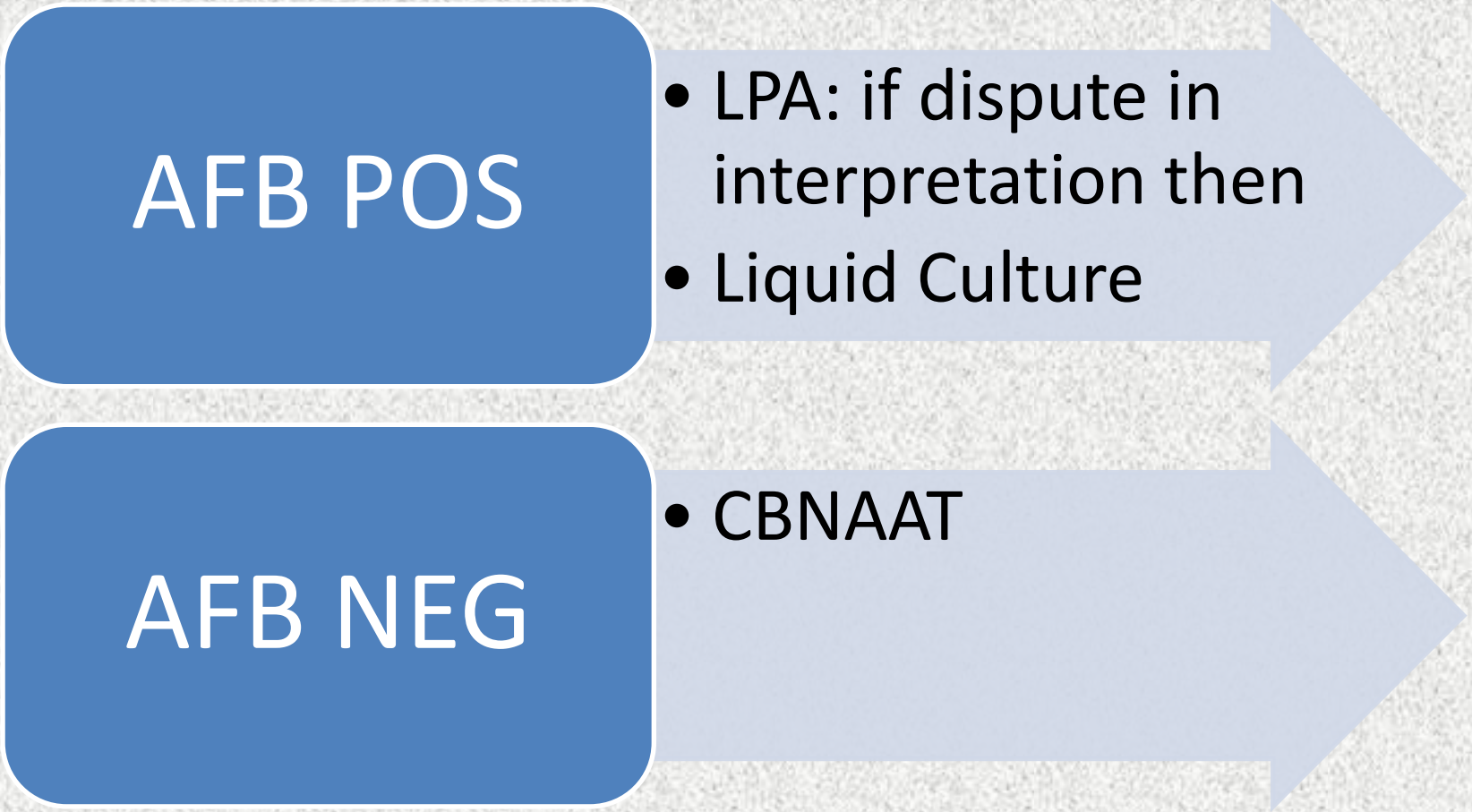
2. A case of Pulmonary TB treated in past

- 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



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

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..

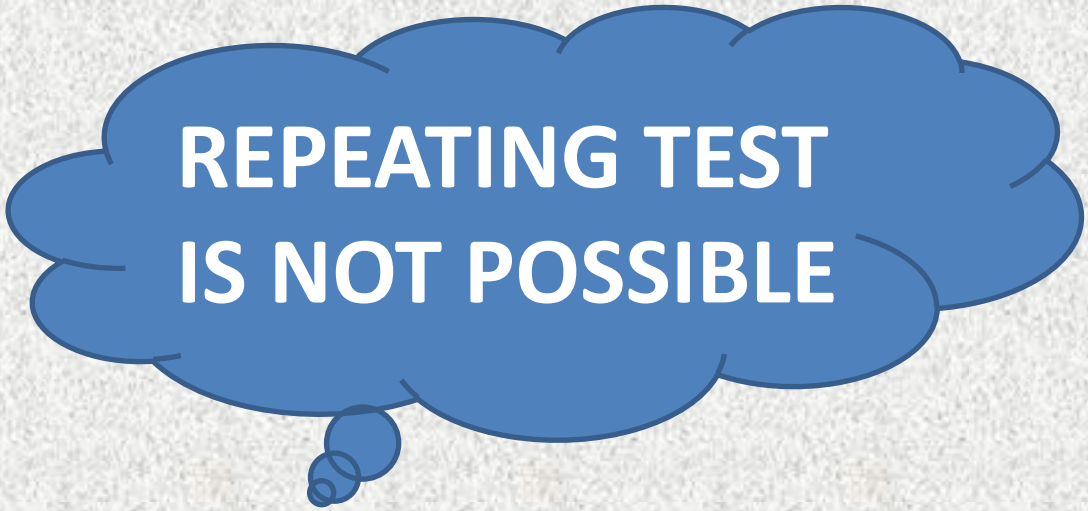
- Quantity: As much as possible
- Quality: Blood contamination as minimum as possible, in screw capped sterile container, do not let the sample dry, No additives
- Transport: ASAP

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



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

RNTCP Request for Culture and Drug Sensitivity Testing

Annexure I

(Required for Culture and DST laboratory to conduct testing; please send copy to District TB Officer)

Patient information

Patient Name:		
Patient Address with landmark		
Patient Mobile No. or other Contact No.		
Age:		Sex: ❑ Male ❑ Female
Sputum – Date of collection (DD/MM/YY):		Sample 1: _____ Sample 2: _____
Name referring facility (PHI/DMC/DR-TB Center / other):		
District		
Tuberculosis Unit (TU)		

Reason for Testing (For SL DST)

❑ DIAGNOSIS	❑ FOLLOW-UP
MDR Suspect Criteria ❑ Failure ❑ Re-treatment case S+ at 4th month ❑ Contact of known MDR TB case ❑ S+ at diagnosis, re-treatment case ❑ Any follow up S+ ❑ S- at diagnosis, re-treatment case ❑ HIV TB case	PMDT Registration Number Treatment month of Follow-up
RNTCP TB Reg No. and Type (or ❑ Not Applicable)	DR-TB Centre Name

Molecular TB/DST result

Test	❑ Line Probe Assay (LPA)	❑ CBNAAT	
Test validity:	❑ Valid	❑ Invalid	
M. Tuberculosis	❑ Detected	❑ NOT detected	
Rifampicin:	❑ Resistant	❑ Sensitive	❑ Not Available
Isoniazid:	❑ Resistant	❑ Sensitive	❑ Not Available

Notes:

Date tested: Reported by (Name & Signature):

LJ/ Liquid Culture results

Date received	Specimen	Specimen No.	Smear result	Culture Result* (check one)						
				Neg	Pos	1 - 19 col	+	+ +	+ + +	Contaminated/ Other result
	A									
	B									

Notes:

Result Date: Reported by (Name & Signature):

LJ/ Liquid culture DST Results: (Note: 'S' if susceptible , 'R' if resistant)

Date DST Initiated	Specimen No	S	H	R	E	Z	Km	Ofx	Eto	Other

Result Date: Reported by (Name & Signature):

If SL-DST is desired please mention

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

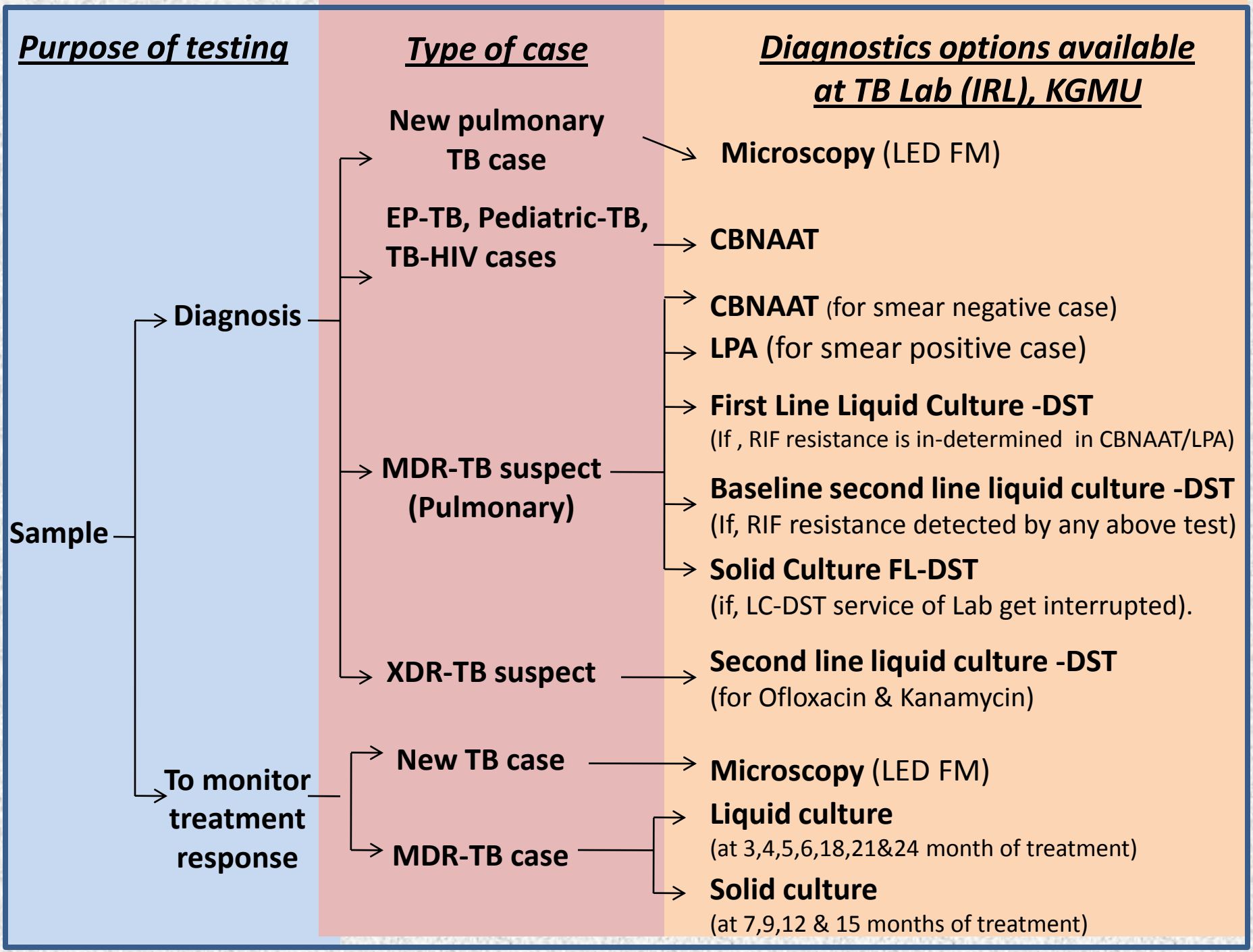
Detection of TB and DR TB: Issues with endemic countries

- 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
Diagnostics options available at TB Lab (IRL), KGMU
Sample
Diagnosis
To monitor treatment response
New pulmonary TB case
Microscopy (LED FM)
EP-TB, Pediatric-TB, TB-HIV cases
CBNAAT
MDR-TB suspect (Pulmonary)
CBNAAT (for smear negative case)
LPA (for smear positive case)
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).
XDR-TB suspect
Second line liquid culture -DST
(for Ofloxacin & Kanamycin)
New TB case
Microscopy (LED FM)
MDR-TB case
Liquid culture
(at 3,4,5,6,18,21&24 month of treatment)
Solid culture
(at 7,9,12 & 15 months of treatment)

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RNTCP

FIND

GLOBAL FUND

Thank You