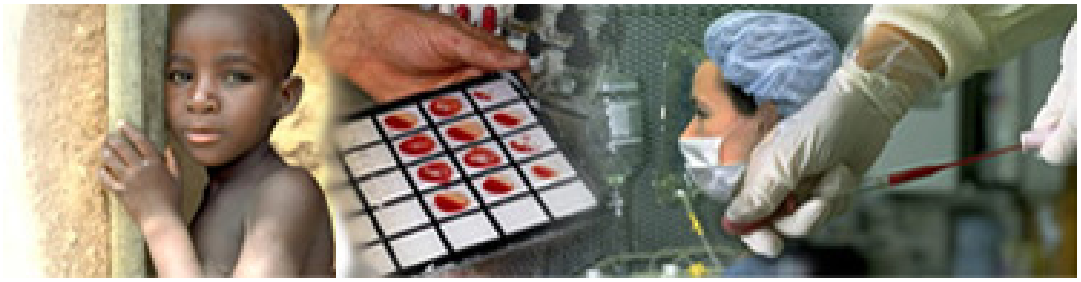

The reference standard for evaluating molecular assays

Advanced TB Diagnostic Research
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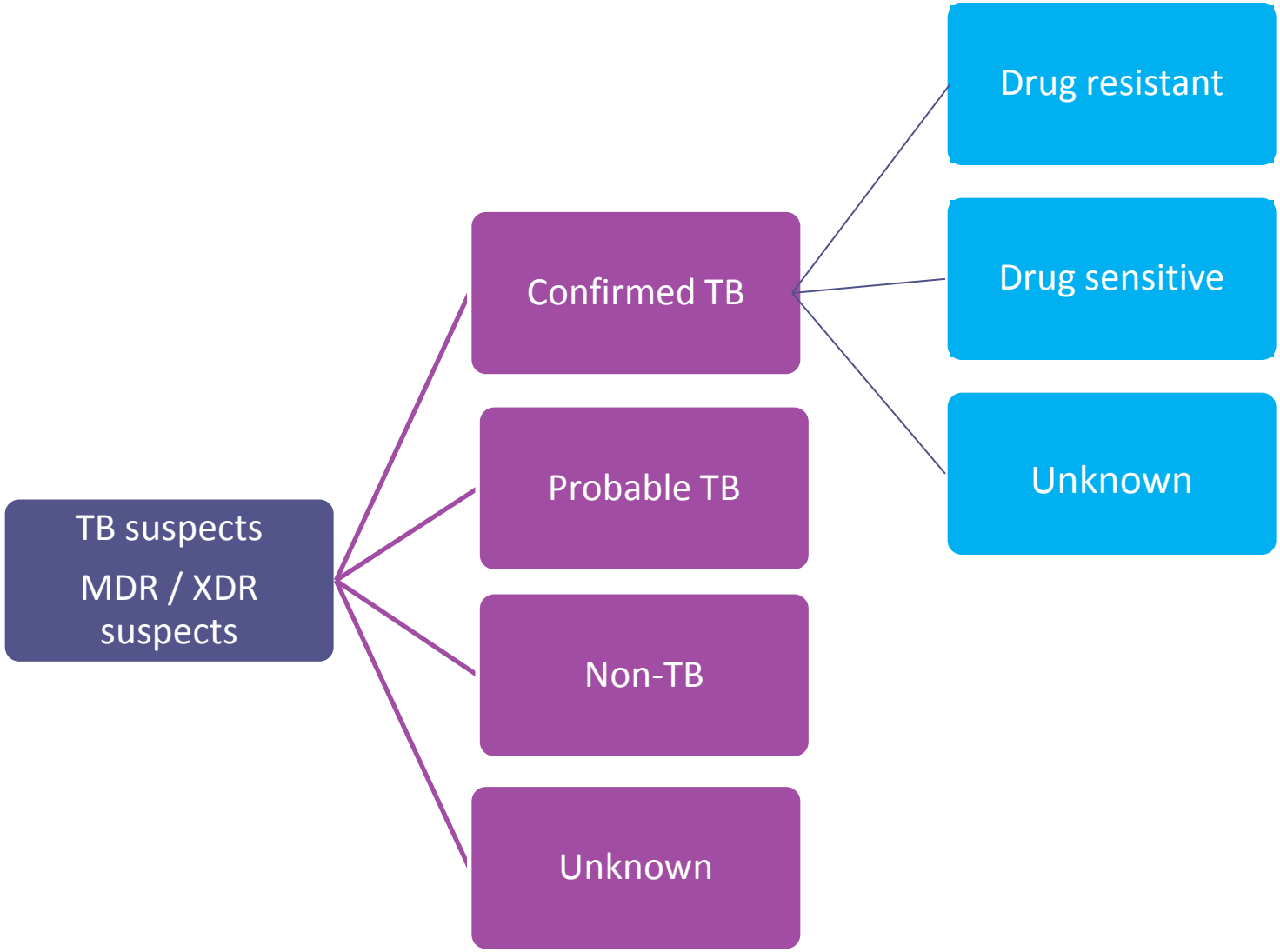
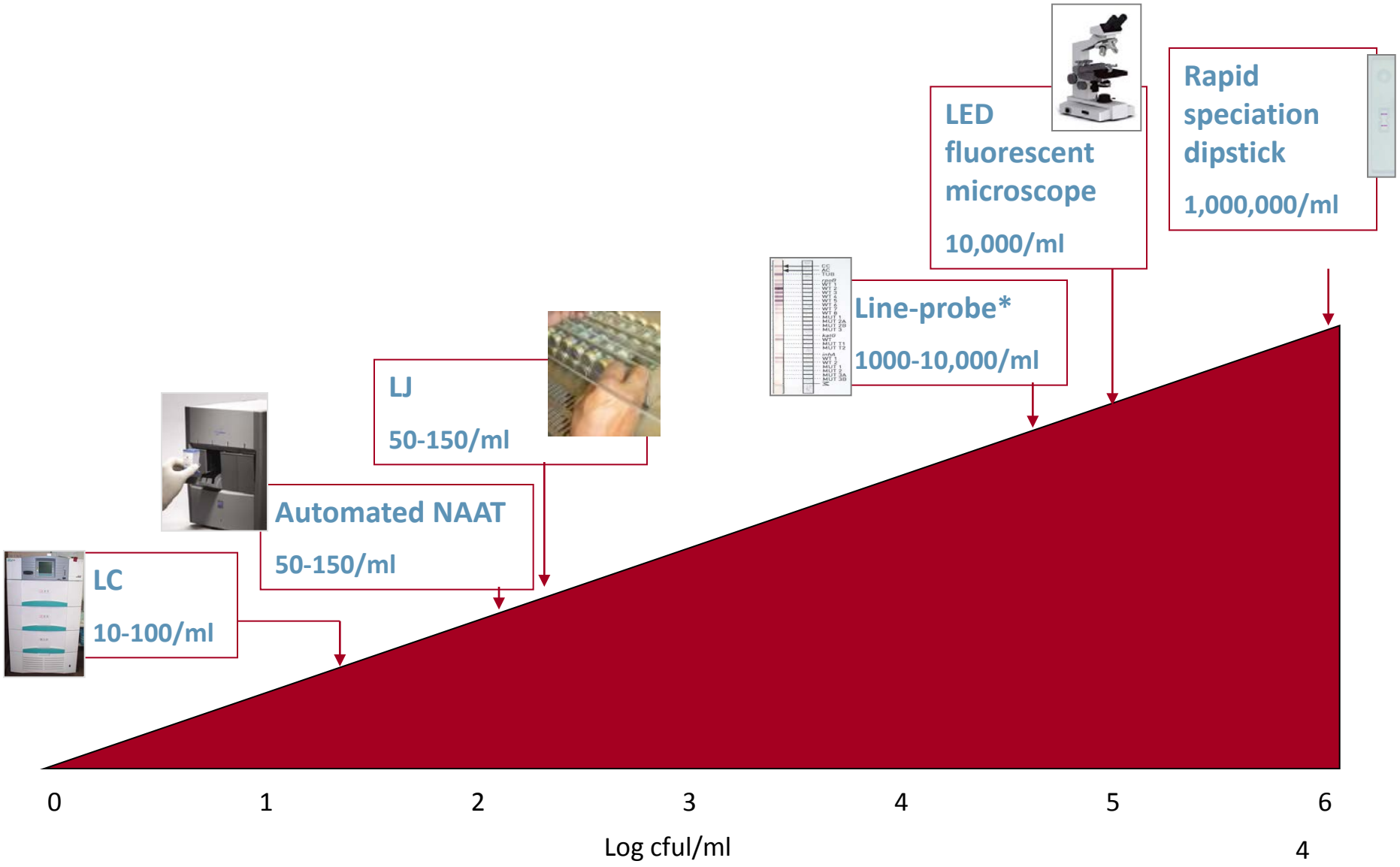
Key groups for analysis in an accuracy study for a new molecular test



Possible reference standard methods

1. Smear microscopy	**Ziehl-Neelsen** **FM; LED-FM**
2. Solid Media **(Proportion, Resistance Ratio, Absolute Concentration)**	**Löwenstein-Jensen** **Middlebrook** **Ogawa**
3. Liquid Media	**BACTEC 460** **MGIT & MGIT 960** **MB BacT**
4. Alternative culture methods	**MODS** **Nitrat Reductase** **Colorimetric Redox Indicators** **TLA**
5. Molecular Technics	**GenoType® MTBDRplus & SL** **INNO-LiPA Rif.TB** **Xpert MTB/RIF** **Other NAAT / Sequencing**

Sensitivity (cfu/ml) of pulmonary TB tests in portfolio



LC Demonstration Projects (MGIT)

Higher rate of mycobacterial isolation

- Of 2458 specimens, 54% were culture-pos by MGIT & 39% by LJ
- Of 608 smear-neg, culture-pos specimens, 94% pos by MGIT & 41% by LJ

Feasibility of MGIT only

- Of 1423 specimens with any pos culture, MGIT was pos in 93% & LJ in 67%

Shorter time to detection

- Median time to detection 9 d with MGIT & 28 d with LJ
- The median time to DST result was 9 d (6-12) with MGIT & 21 d (18-21) with LJ

High concordance between MGIT SIRE and LJ DST results

- 98% for R and H, 96% for E, 92% for S

Higher contamination rate & frequent detection of NTM

- Initially > 20%, but can be decreased to ≤10% over time
- 8% of MGIT cultures and 2% of LJ cultures were contaminated
- (Rapid) Species identification required for all positive cultures
- Rapid growing NTM outgrow MTB, risk of missing TB diagnosis (advantage of adding LJ)
- Mixed strains impact on DST

TB species confirmation from culture

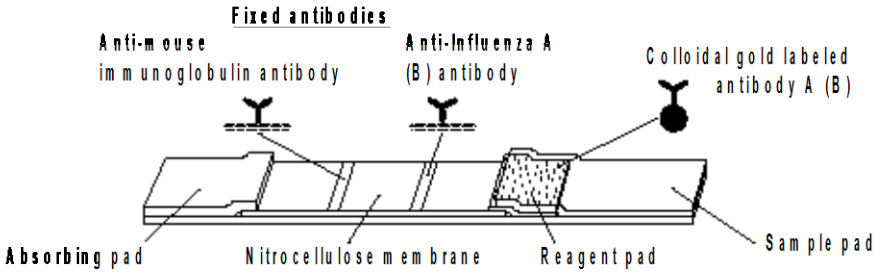
1. **Conventional biochemical reaction,**

- Gold standard, but quality depending on lab tech skills
- Variable quality of PNB rapid tests

2. **Genotype MTB**

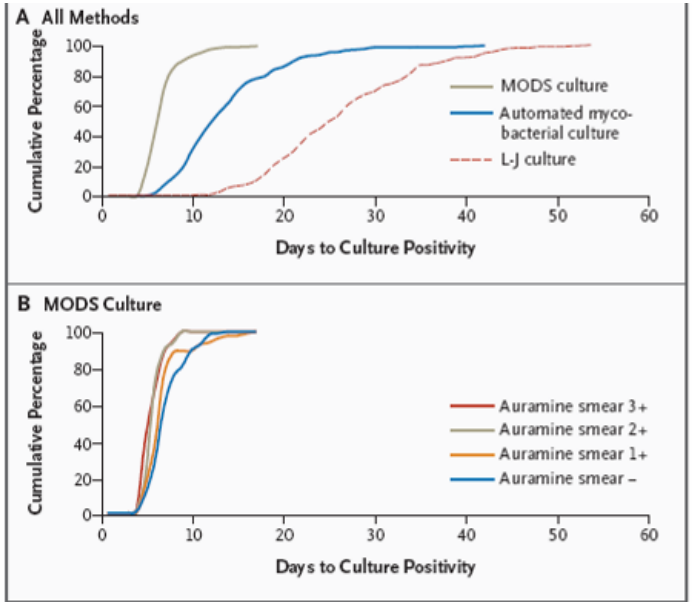
3. **MPT64 antigen tests (Tauns; SD; BD)**, specific for *M. tuberculosis* complex

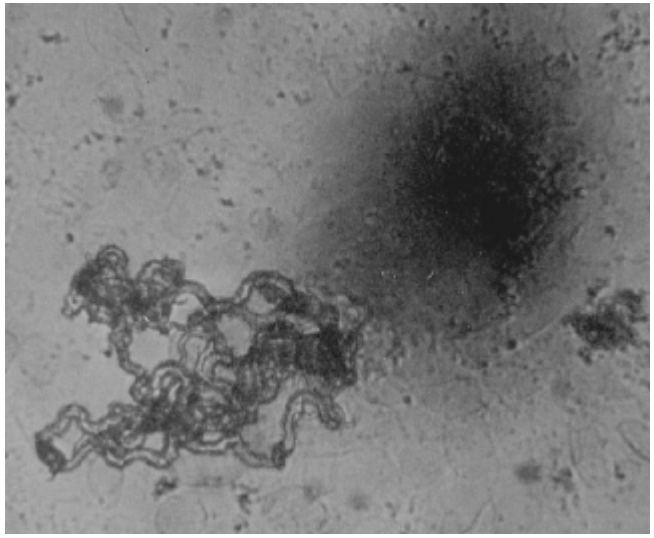
- Capilia TB superior (SD – less sensitive; BD – false positivity), but not widely available
  - Evaluated in >20,000 tests in >15 countries
  - Very high sensitivity and specificity compared to standard biochemical detection
  - Cross-reaction with *M. marinum* excluded for new generation, sensitivity increased compared to first generation.
  - All tested MGIT cultures Capilia positive already at day of MGIT instrument positivity



Microscopic Observation Drug Susceptibility (MODS)

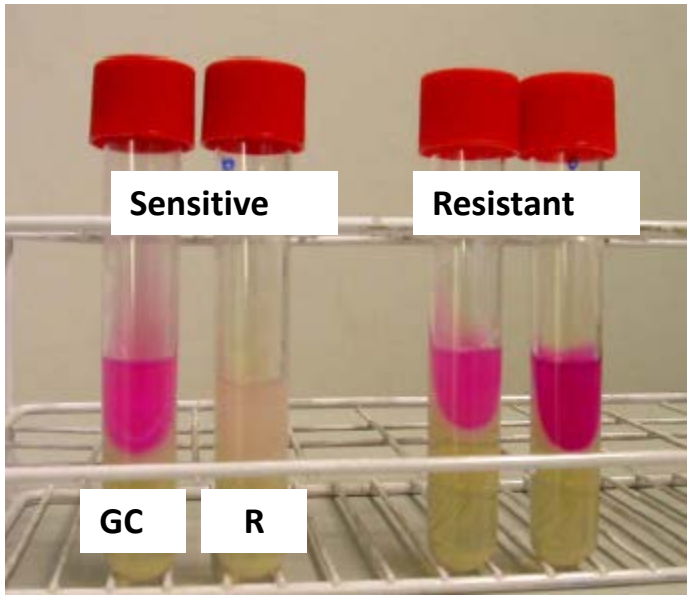
- Caviedes et al. JCM 2000
- Relies on 1. Characteristic "cording"
  2. Rapid growth in Middlebrook 7H9 broth
- Moore et al. NEJM 2006:
  - MTB detection rapid (median 7 d; 13 d MBBacT and 26 d LJ)
  - Sensitive (98% vs. 89% vs. 84%).
  - High concordance for direct R & H DST w median time to detection of 7 d.





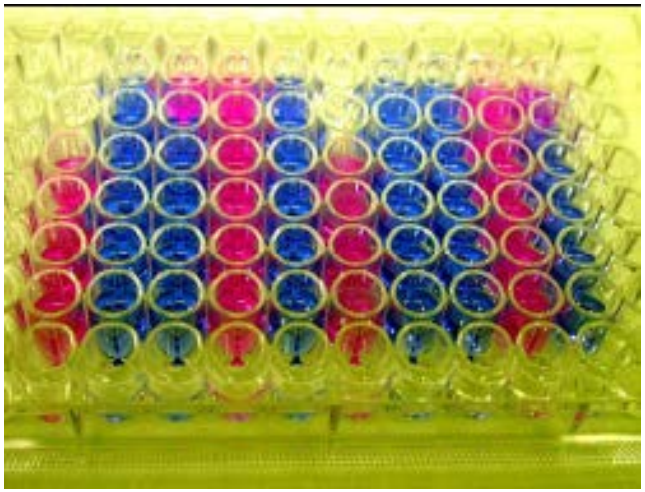
Nitrate Reductase Assay (Griess method)

- Based on ability of MTB to reduce nitrate to nitrite
- Ängeby et al. JCM 2002 (indirect)
- Affolabi et al. JCM 2007 (direct method for smear pos)
- Time to detection 10 d (indirect); 14 – 28 d (direct)
- Promising sensitivity and specificity for R and H
- Caveats: specificity?



Colorimetric Redox Indicators

- Based on reduction of redox indicators by mycobacterial metabolism
- Alamar Blue (Yaiko et. al JCM 1995); Resazurin (Palomino et al. AAC 2002)
- Meta-analysis Martin et al. JAC 2006: sensitivity and specificity range for R and H 89-100%
- Caveat: Aerosols and possibility of cross contamination



Is a single culture as reference standard sufficient?

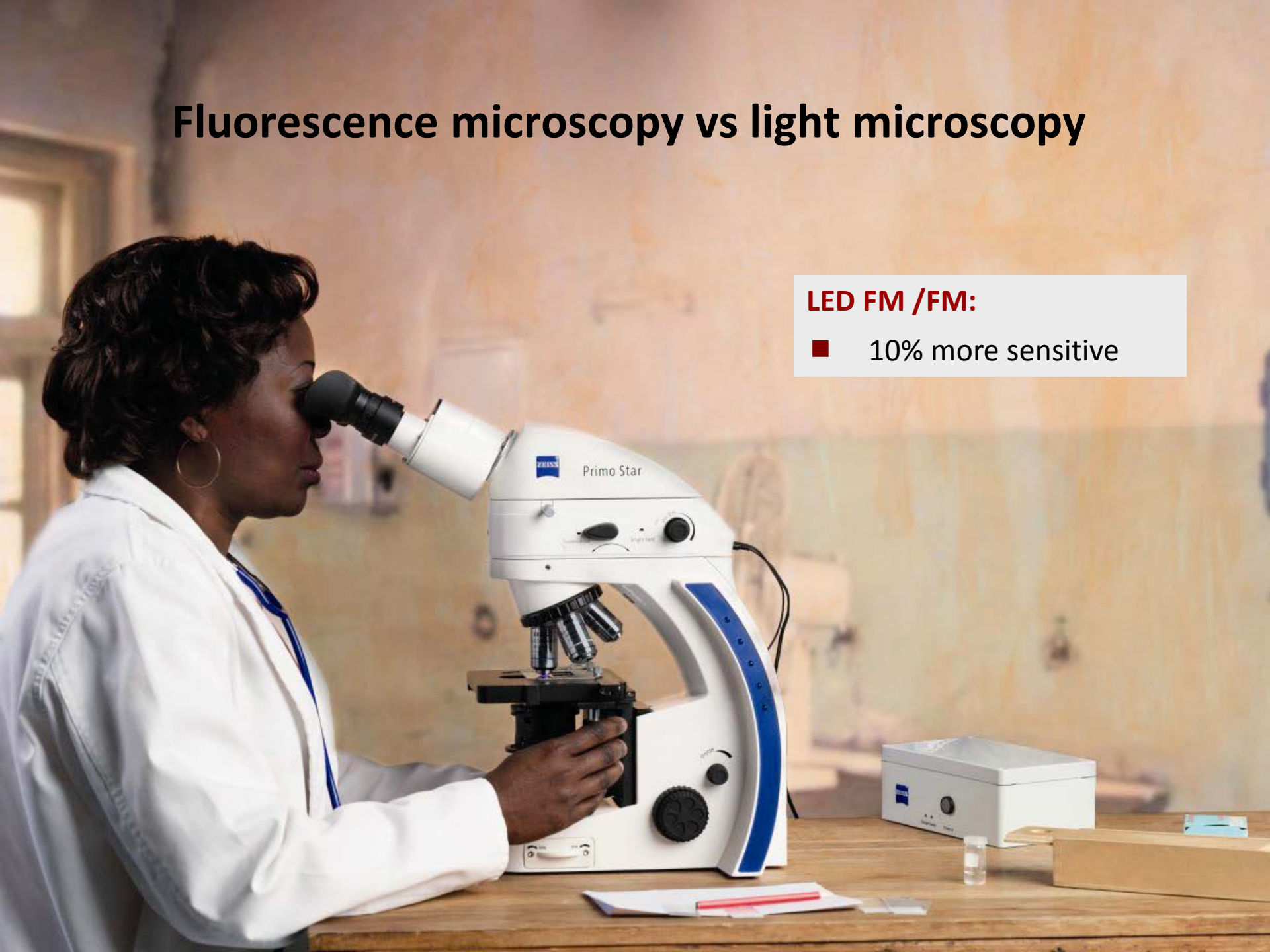
Table : Comparison of the overall sensitivity of a single LJ culture, a single MGIT culture and a single, direct Xpert MTB/Rif test using the results of 3 smears and 4 cultures per patient as a reference standard.

Patient group	Single LJ*	Single MGIT*	Single, direct Xpert †
Smear-positive, Culture-positive	93.0% (1031/1109)	97.7% (1106/1132)	98.2% (551/561)
Smear-negative, Culture-positive	69.4% (222/320)	84.5% (283/335)	72.5% (124/171)
All Culture-positive	87.7% (1253/1429)	94.7% (1389/1467)	92.2% (675/732)

Performance targets met? It depends…..

Reference standard		Sensitivity in C+	Sensitivity in S+C+	Sensitivity in S-C+	Specificity in S-C-
2 conc smears, 2 direct smears & 2 LJ + 2 MGIT cultures	Test X	83.7% (340/406) [79.8%-87.0%]	97.8% (262/268) [95.2%-99.0%]	56.5% (78/138) [48.2%-64.5%]	90.2% (517/573) [87.5%-92.4%]
2 direct smears & 2 LJ + 2 MGIT cultures	Test X	83.7% (340/406) [79.8%-87.0%]	97.7% (214/219) [94.8%-99.0%]	67.4% (126/187) [60.4%-73.7%]	90.2% (515/571) [87.5%-92.4%]
2 direct smears & 2 LJ	Test X	88.3% (326/369) [84.7%-91.2%]	97.7% (209/214) [94.6%-99.0%]	75.5% (117/155) [68.1%-81.6%]	89.7% (533/594) [87.0%-91.9%]

Fluorescence microscopy vs light microscopy



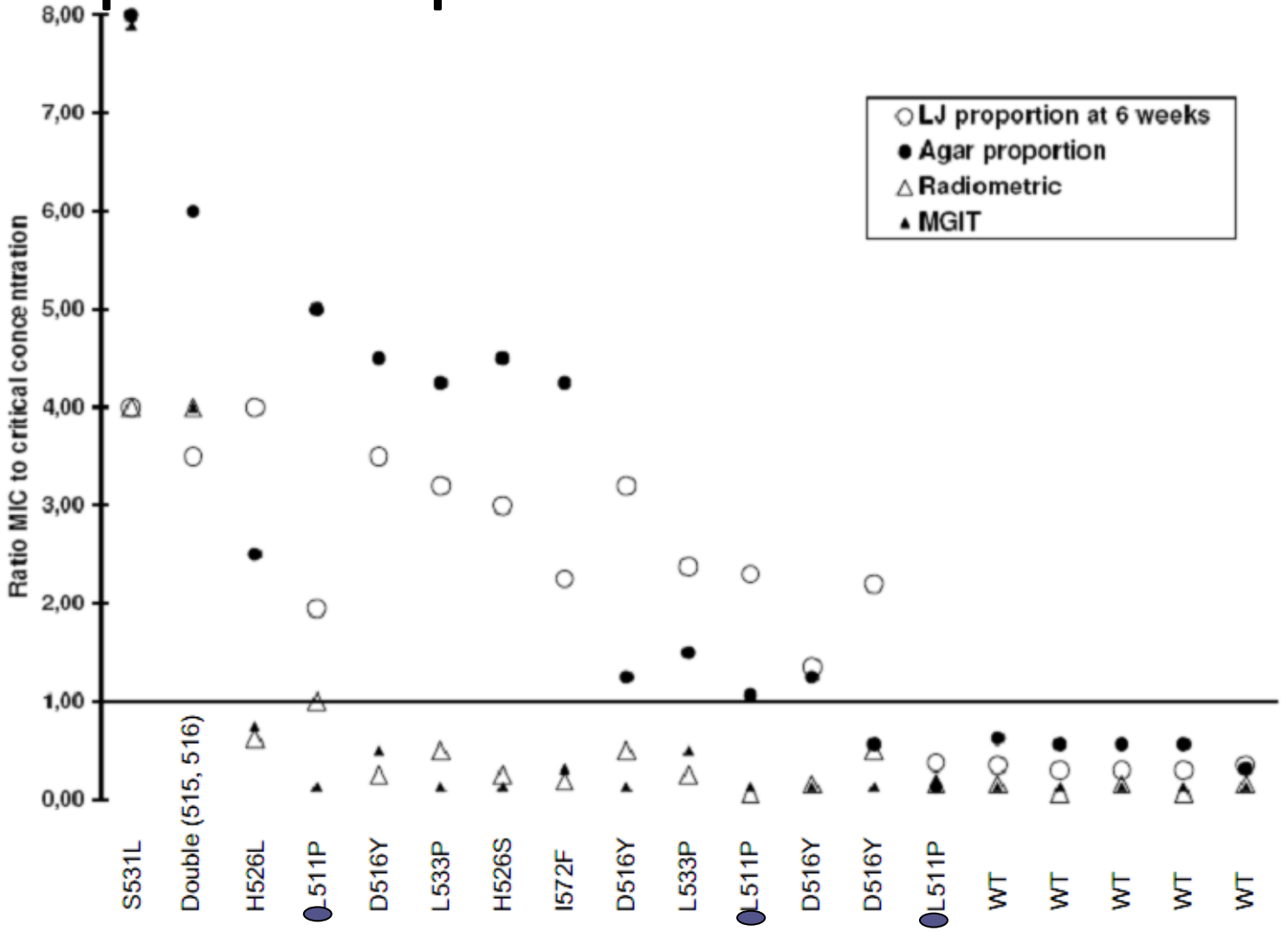
LED FM /FM:

- 10% more sensitive

Nucleic acid amplification tests

Assay	Author	n	Sens/Spec	Smear + Sens	Smear - Sens
Gen Probe	Abe	135	92/100	100	77
	Miller	594	91/99	100	73
	Pfyffer	515	94/98	100	83
	O'Sullivan	555	91/99	100	75
Roche	D'Amato	985	67/100	95	55
	Wobeser	1480	79/99	98	53
	Carpentier	2073	86/98	95	74
	Moore	1009	83/99	99	66
	Bergman	956	79/100	98	43
	Ichyama	530	89/100		
BD	Ichyama	530	95/99.8		
	Pfyffer	799	98/96.5	100	92

Culture methods can have difficulty identifying specific rifampin-resistant mutants



[Adapted from] Wright et Al. *Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results.* J Clin Microbiol. 2009 Sep 16. [Epub ahead of print]

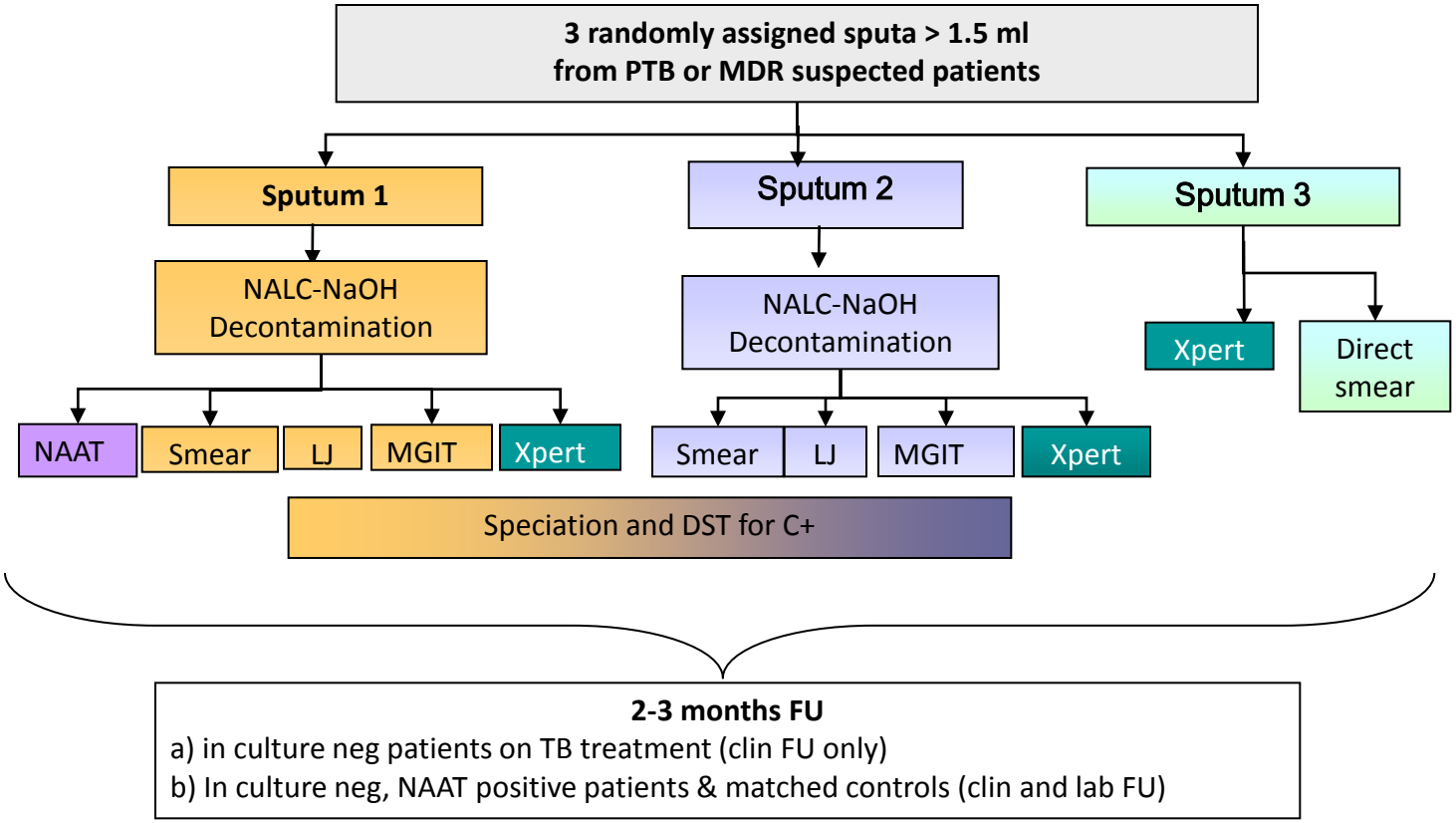
Subgroup analysis for culture negative MDR suspects on TB treatment at enrolment

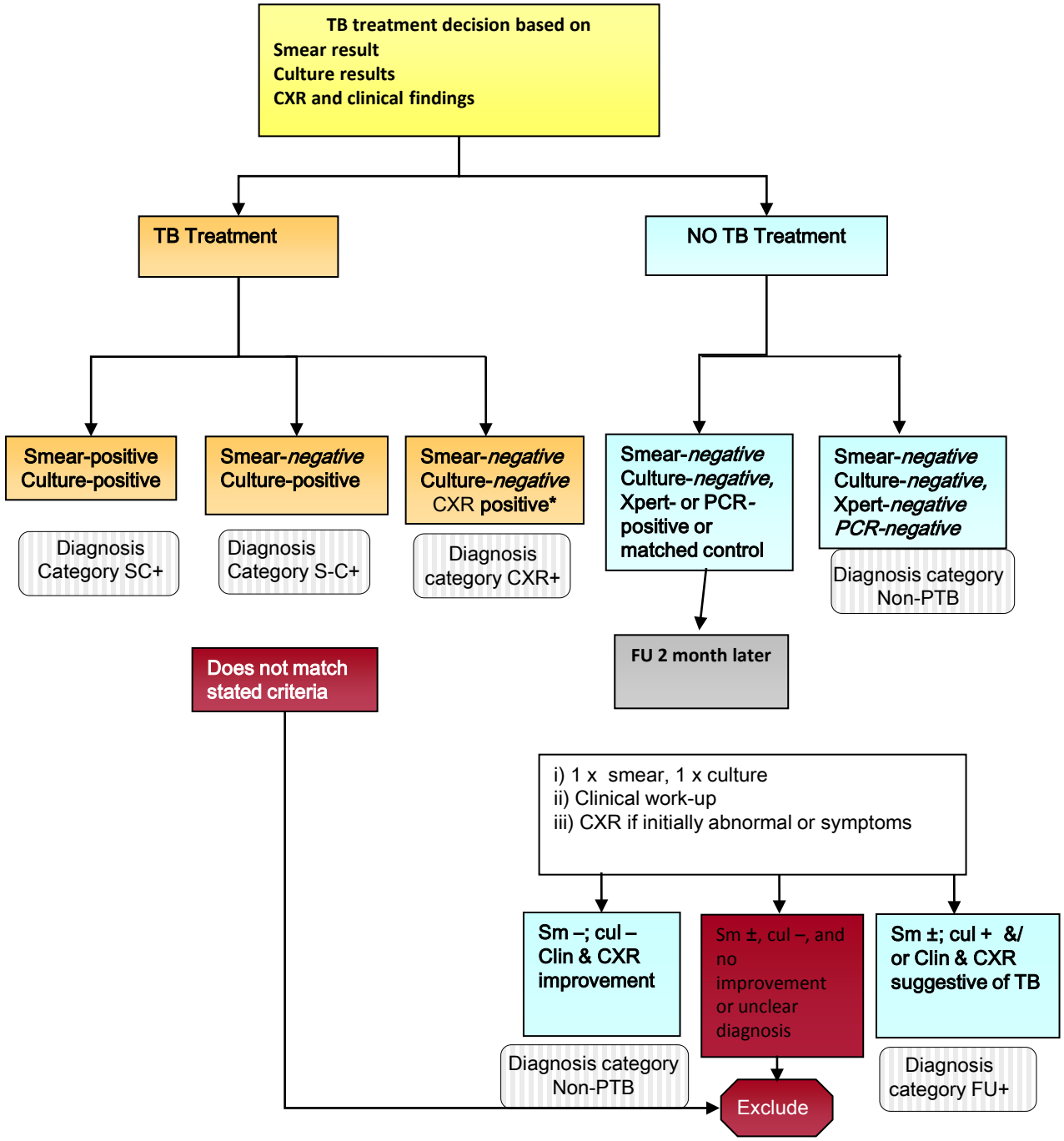
	Total	UPCH	STI	UCT	SAMRC	Hinduja
Xpert positive, %, (positive/total)	**44.3 (51/115)**	50.0 (1/2)	49.0 (25/51)	66.7 (2/3)	50.0 (5/10)	37.0 (18/49)
Xpert RMP-resistant, %, (resistant/total)	**15.7 (8/51)**	0.0 (0/1)	8.0 (2/25)	0.0 (0/2)	40.0 (2/5)	22.2 (4/18)
% of these started on MDR TB treatment for clinical reasons, (started/total)	**100% (8/8)**	NA	100.0 (2/2)	NA	100.0 (2/2)	100.0 (4/4)

How to deal with the following groups:

- No valid index test result
- No valid culture
- Single pos culture <20 colonies in SC or >28 days in LC
- S+C-
- Discrepant conventional DST results
- No MTB confirmation (NTM)
- Culture pos at FU only
- Clinical diagnosis of TB in S-C-
- Culture negative patients on TB treatment

Xpert evaluation studies




TB treatment decision based on
Smear result
Culture results
CXR and clinical findings
TB Treatment
NO TB Treatment
Smear-positive Culture-positive
Smear-negative Culture-positive
Smear-negative Culture-negative CXR positive*
Smear-negative Culture-negative, Xpert- or PCR-positive or matched control
Smear-negative Culture-negative, Xpert-negative PCR-negative
Diagnosis Category SC+
Diagnosis Category S-C+
Diagnosis category CXR+
Diagnosis category Non-PTB
FU 2 month later
Does not match stated criteria
i) 1 x smear, 1 x culture
ii) Clinical work-up
iii) CXR if initially abnormal or symptoms
Sm –; cul – Clin & CXR improvement
Sm ±, cul –, and no improvement or unclear diagnosis
Sm ±; cul + &/ or Clin & CXR suggestive of TB
Diagnosis category Non-PTB
Diagnosis category FU+
Exclude

Reference standard for

1. **Case detection**
   - Min: 1 LC/SC with speciation / Opt: LC/SC from 2 specimens with speciation
   - FU: Min: Discrepant/controls / Opt: All Non-TB & Clin TB
   - Caveat: Patients on TB treatment (exclusion criteria, subanalysis)
   - Caveat: NTM / mixed infections
   - Option: Add WRT such as Xpert as additional comparator (subanalyses)
   - Probable TB / Clinical TB: FU if needed
2. **Drug resistance detection**
   - Min: 1 phenotypic test; Opt: phenotypic and sequencing of all (separate analysis)