

The reference standard for evaluating molecular assays

Advanced TB Diagnostic Research

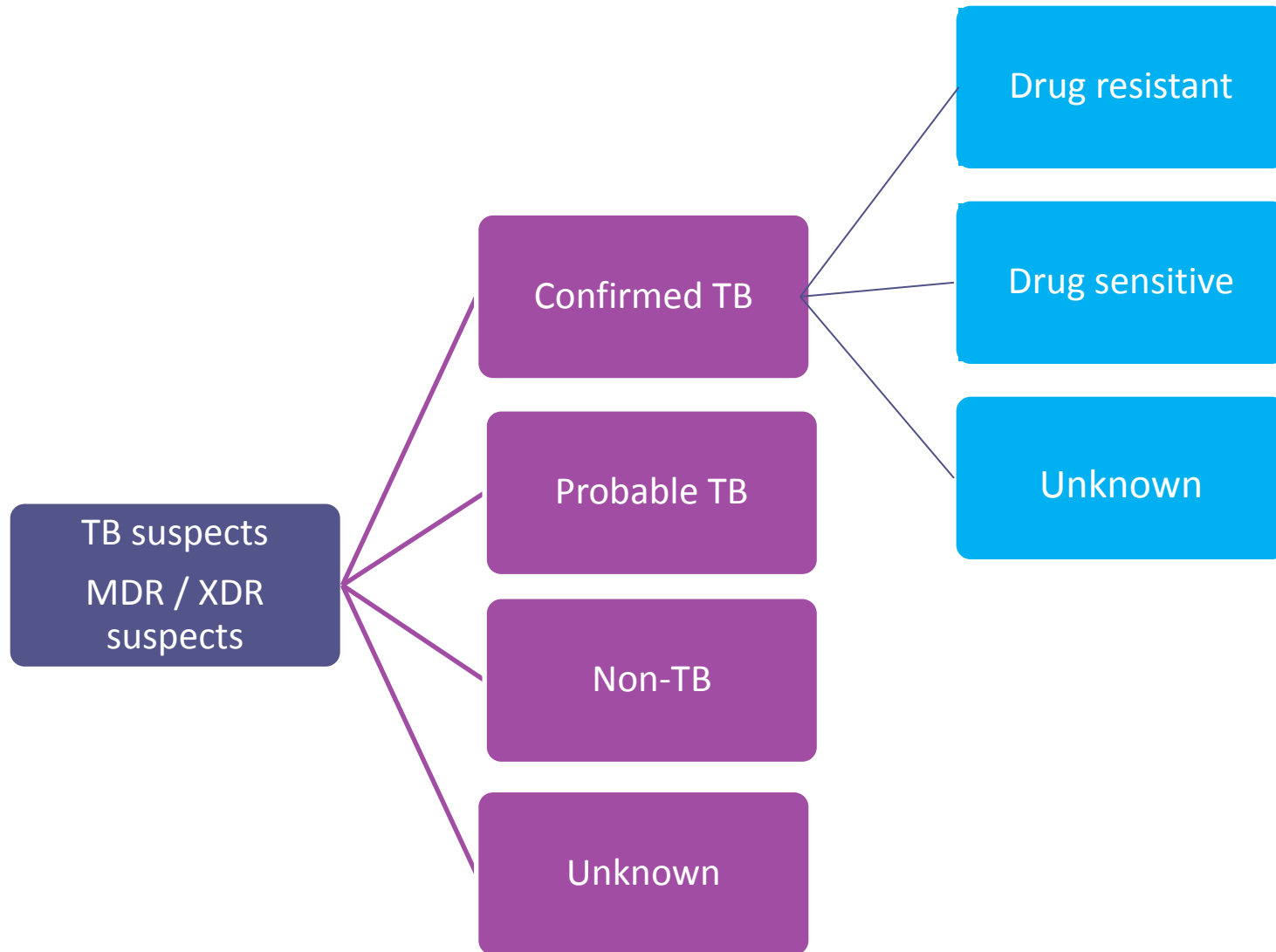
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Partnering for better diagnosis for all

Key groups for analysis in an accuracy study for a new molecular test



Problem 1: Suboptimal sensitivity of microbiological gold standard

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 in S-C+ 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	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	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	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%]

Possible microbiological reference standard methods

1. Smear microscopy

Ziehl-Neelsen

2. Solid Media

FM; LED-FM

(Proportion, Resistance Ratio, Absolute Concentration)

Löwenstein-Jensen

Middlebrook

3. Liquid Media

Ogawa

BACTEC 460

MGIT & MGIT 960

4. Alternative culture methods

MB BacT

MODS

Nitrat Reductase

Colorimetric Redox

Indicators

5. Molecular Technics

TLA

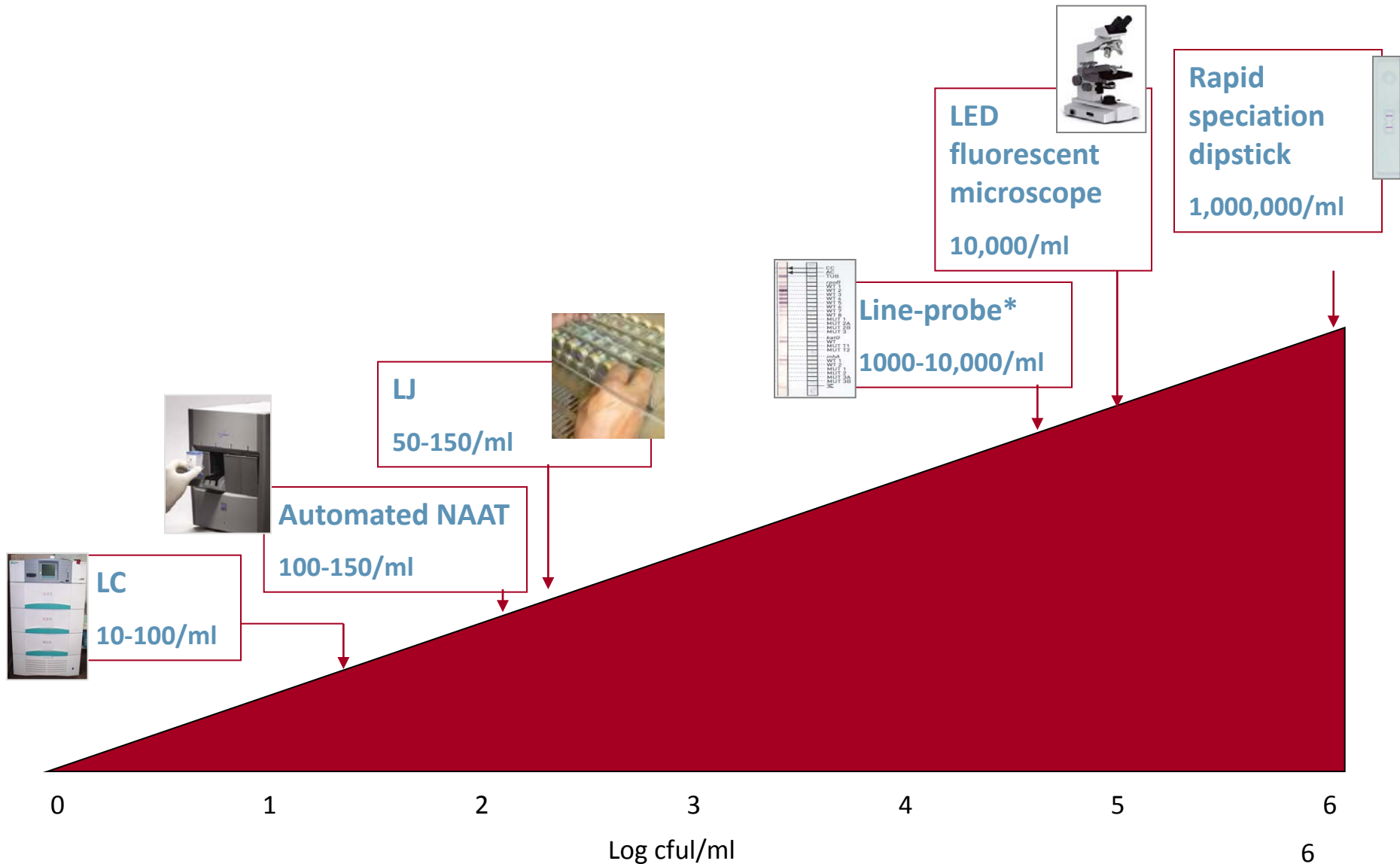
GenoType® MTBDRplus & SL

INNO-LiPA Rif.TB

Xpert MTB/RIF

Other NAAT / Sequencing

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



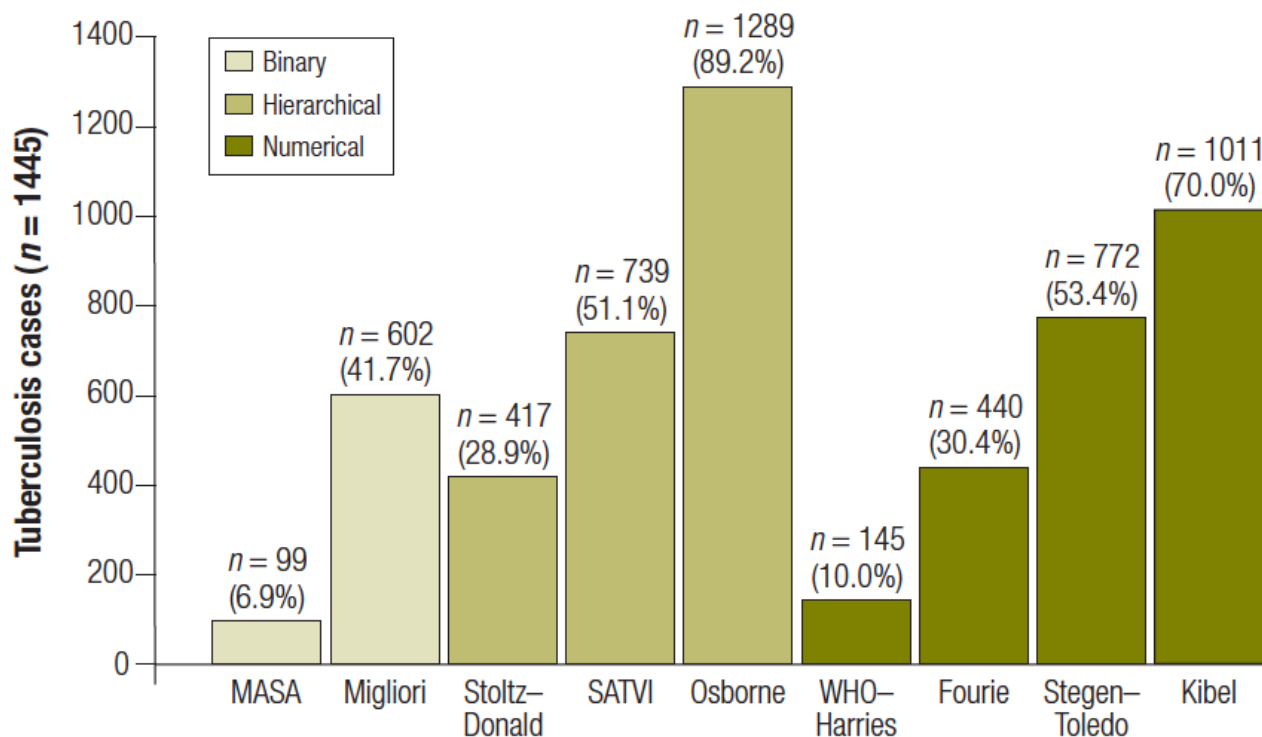
Nucleic acid amplification tests

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

Problem 2: The definition of probable TB

Example: Interpretation of CXR is highly inconsistent

Fig. 1. Frequency of cases classified as tuberculosis with various scoring systems, with hierarchical and numerical outcomes condensed to a binary "tuberculosis/not tuberculosis" output, South Africa, 2001–2006



MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

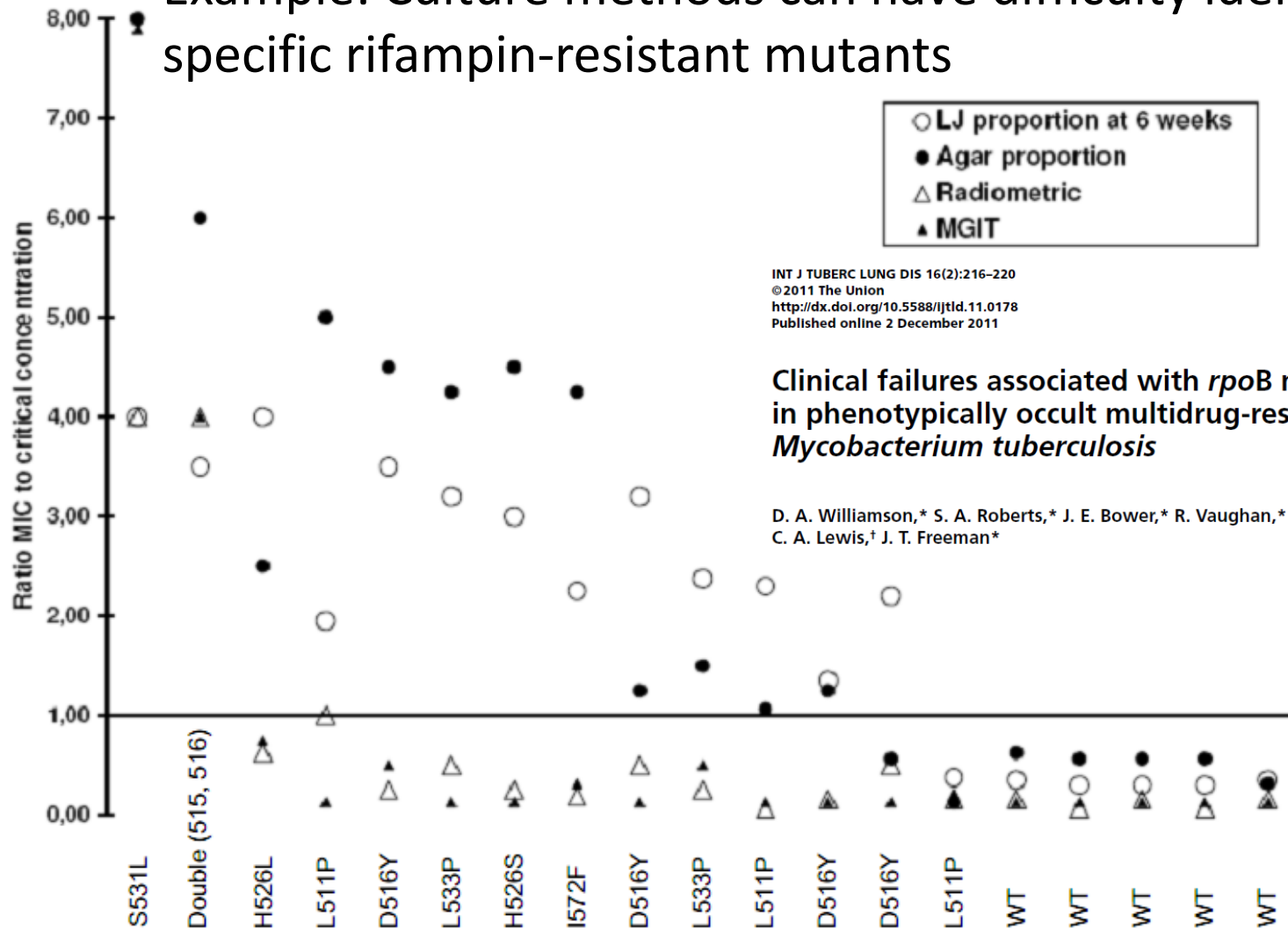
Problem 3: Patients on TB treatment

Example: 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)

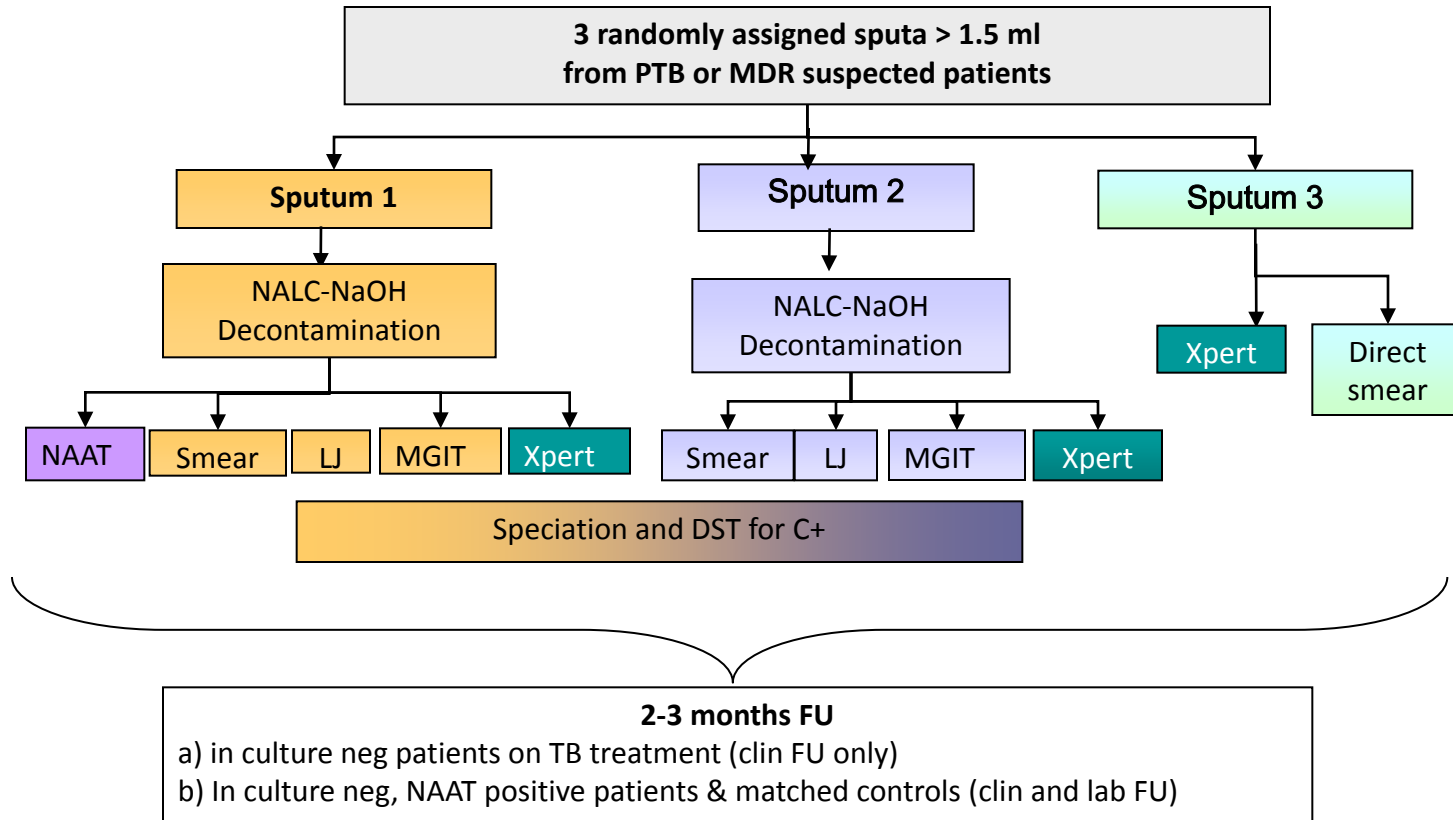
Problem 4: Phenotypic vs genotypic

Example: Culture methods can have difficulty identifying specific rifampin-resistant mutants

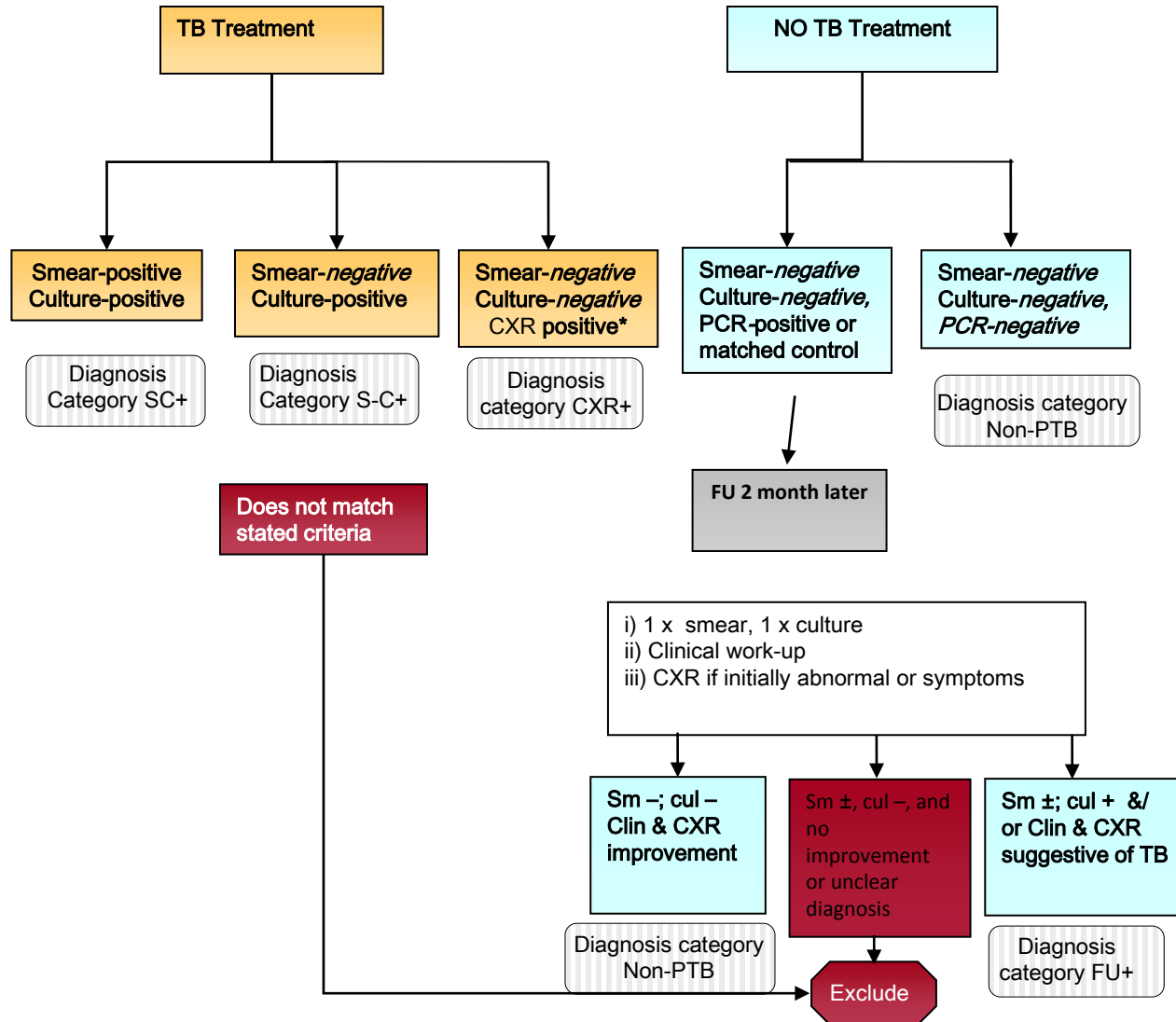


[Adapted From] Wright et Al. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. J Clin Microbiol. 2009 Sep 16. [Epub ahead of print]

Problem 5: Variability of sputum quality



Problem 6: Culture neg, PCR pos patients



The tricky groups for analysis

- ❖ 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
- ❖ NTM vs mixed culture
- ❖ Culture pos at FU only
- ❖ Clinical diagnosis of TB in S-C-
- ❖ Culture negative patients on TB treatment

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)