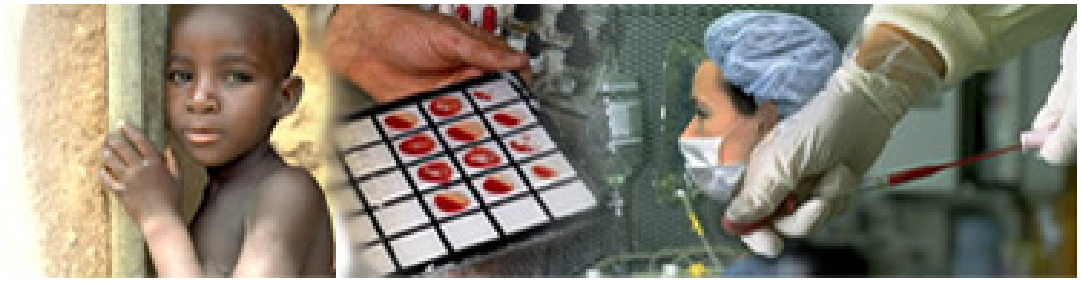
foundation
for innovative new diagnostics



Reference standard for evaluating molecular assays

Advanced TB Diagnostic Research
Montreal, July 9 2013
Pamela Nabeta

Partnering for better diagnosis for all

Disclosure

- FIND is a non-profit foundation devoted to developing diagnostic tools for poverty-related diseases.
- In this role, FIND has development partnerships with industry, including some manufacturers of products mentioned in this talk.
- FIND has no financial benefits in any form.

Beyond Xpert – next generation molecular tools

Q-POC, QuantuMDx

TrueLab, Molbio

GeneDrive, Epistem

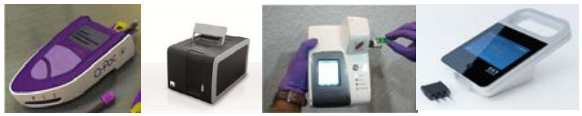
Alere Q

Great Basin Scientific, US

Enigma, UK

Gentura Dx, US

Sensitivity (cfu/ml) of WHO recommended tests



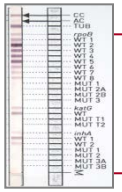
Fast followers

LED fluorescent microscope

10,000cfu /ml

Rapid speciation dipstick

1,000,000cfu/ml



Line-probe*

1000-10,000cfu/ml

LJ/alternative

50-150cfu/ml

Automated NAAT

100-150cfu/ml

LC

10-100cfu/ml

0 1 2 3 4 5 6

Log cful/ml

4

Issue #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)

Single culture may not be sufficient; ideally use 2 LC from 2 different samples. Other issues: culture contamination rate

Issue #2: 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%]

Stringent reference standards should be applied.

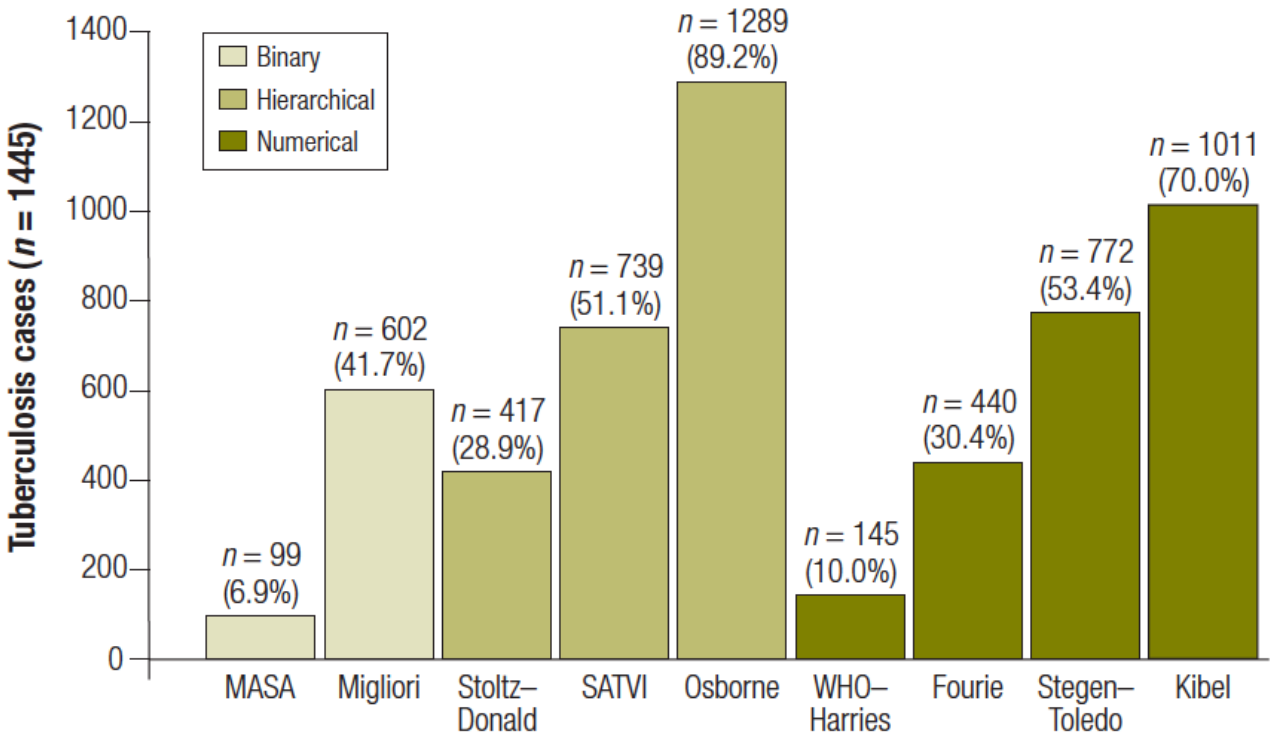
Issue #3: Results comparability

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

Ideally harmonize use of reference standards; include Xpert MTB/RIF

Issue #4: The definition of probable TB

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**

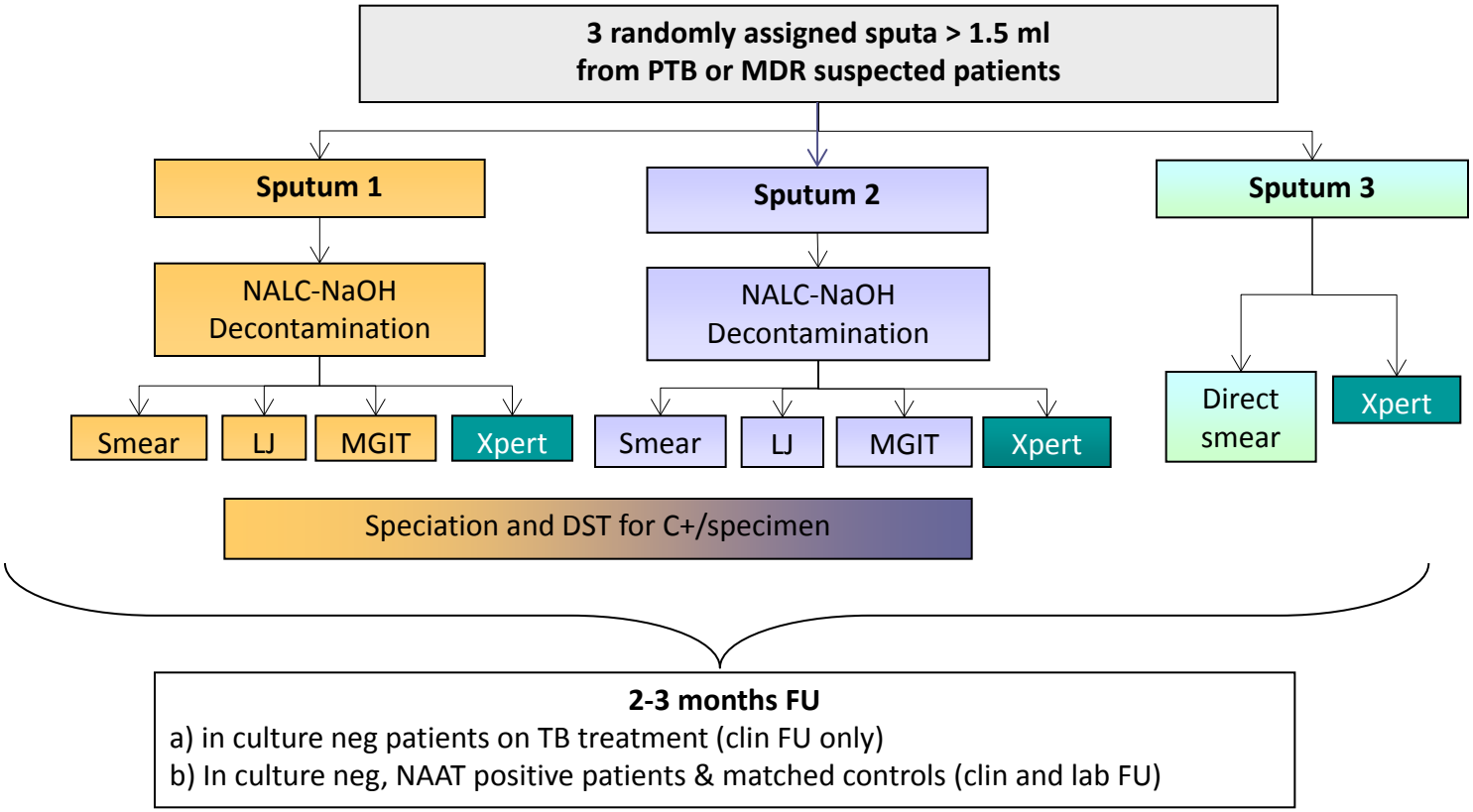


MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

Interpretation of clinical TB is highly inconsistent; consider FU.

Hatherill M et al. Bull World Health Organ 2010;88:312-320

Issue #5: Sputum quality



Sputum quality highly variable, careful design of sample workflow

Issue #6: Phenotypic vs. genotypic

Mycobacterium tuberculosis Strains with Highly Discordant Rifampin Susceptibility Test Results[▽]

A. Van Deun,[1]* L. Barrera,[2] I. Bastian,[3] L. Fattorini,[4] H. Hoffmann,[5] K. M. Kam,[6] L. Rigouts,[1] S. Rüsch-Gerdes,[7] and A. Wright[8]

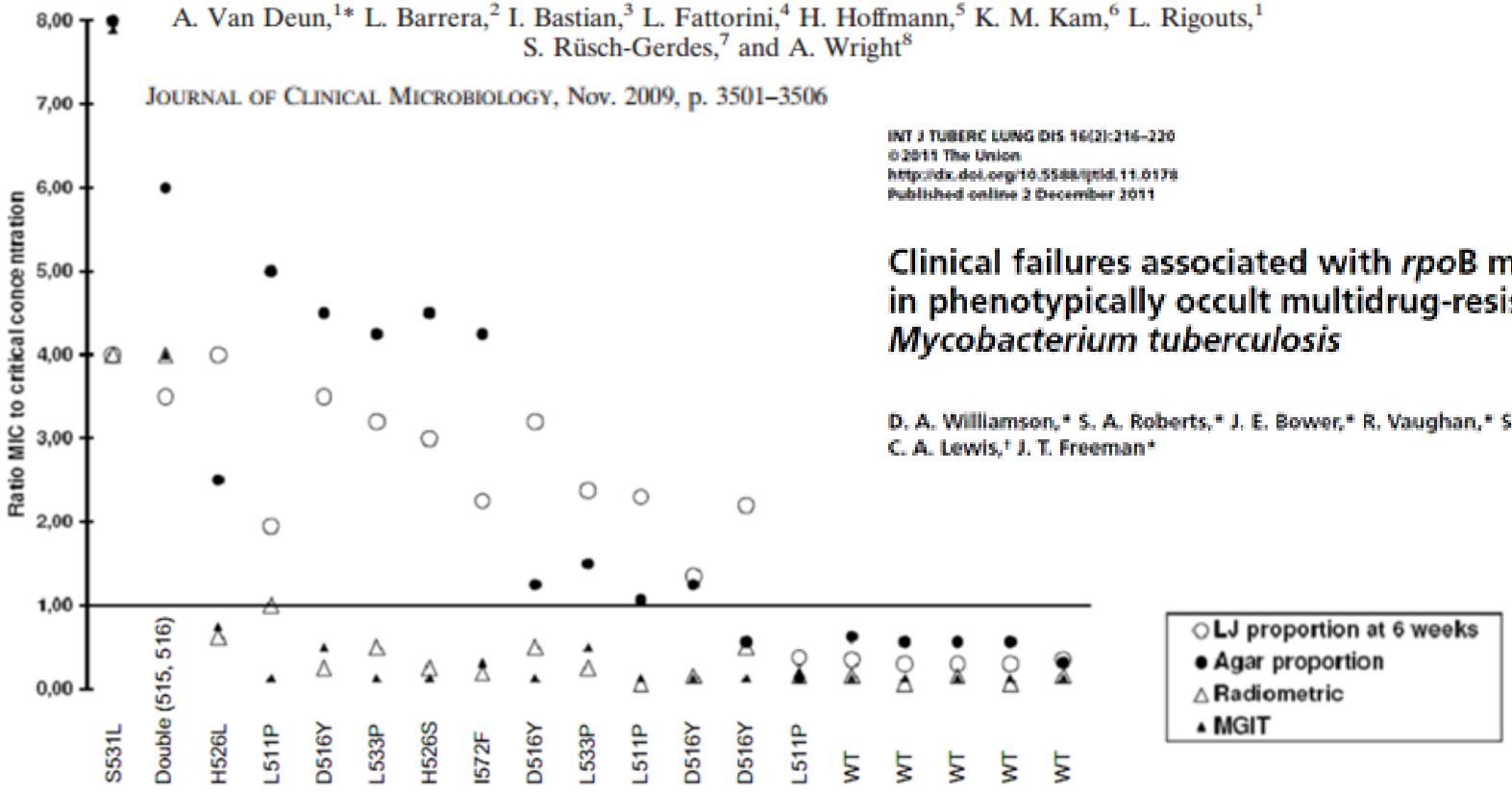
JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3501–3506

INT J TUBERC LUNG DIS 16(2):216–220

http://dx.doi.org/10.5588/ijtld.11.0178
Published online 2 December 2011

Clinical failures associated with *rpo*B mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*

D. A. Williamson,* S. A. Roberts,* J. E. Bower,* R. Vaughan,* S. Newton,* O. Lowe,* C. A. Lewis,† J. T. Freeman*



Culture methods can have difficulty identifying specific Rif-resistant mutants that are clinically relevant. Ideally check for mutations by sequencing.

Issue #7: Detection of drug resistance in 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 Rif- res, %, (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)
% started on MDR TB Tx clinical reasons, (started/total)	**100% (8/8)**	NA	100.0 (2/2)	NA	100.0 (2/2)	100.0 (4/4)

Culture gets negative after treatment initiation. Subgroup analysis.

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 evaluating molecular assays

1. Case detection

- Min→1 LC/SC & speciation / Opt→ LC/SC from 2 specimens & speciation
- FU: Min→Discrepant/controls / Opt→All Non-TB & Clin TB
- Caveats:
  - Patients on TB treatment (exclusion criteria, subanalysis)
  - NTM / mixed infections
- Add Xpert as additional comparator (subanalysis)
- Probable TB / Clinical TB: FU if needed

2. Drug resistance detection

- Min→1 phenotypic test & sequencing of discrepant cases; Opt→ phenotypic and sequencing of all (separate analysis)