

Reference Standards for TB Microscopy Studies

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Why Microscopy?

- Diagnose active TB
- Quick
- Cheap
- Still first choice in many resource limited settings
- Will detect NTMs if present
 - Specificity for TB in endemic countries is high
- No information about susceptibility
- Quantification
 - ? Transmissibility
 - No impact on treatment

Microscopy Sensitivity

Study* (first author, year, country)	FM stain	Number patients or specimens	CM		FM		FM-CM sensitivity	FM-CM specificity
			Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)		
Bennedsen (a), 1966, Denmark ²⁰	AR	372	0.62 (0.57-0.67)	..	0.67 (0.61-0.71)	..	+5%	..
Bennedsen (b), 1966, Denmark ²⁰	AR	372	0.52 (0.47-0.57)	..	0.52 (0.46-0.56)	..	0%	..
Bennedsen (c), 1966, Denmark ²⁰	AR	23 427	0.55 (0.51-0.59)	1.00 (0.99-0.99)	0.63 (0.59-0.66)	1.00 (0.99-0.99)	+8%	0%
Bennedsen (d), 1966, Denmark ²⁰	AR	23 427	0.55 (0.51-0.58)	1.00 (0.99-0.99)	0.58 (0.54-0.62)	1.00 (0.99-0.99)	+3%	0%
Burdash, 1975, USA ⁴¹	AR	250	0.94 (0.78-0.99)	1.00 (0.98-1.00)	0.97 (0.83-0.99)	0.99 (0.96-0.99)	+3%	-1%
Githui, 1993, Kenya ⁴⁶	A	1480	0.65 (0.61-0.67)	0.97 (0.95-0.98)	0.80 (0.77-0.82)	0.96 (0.93-0.97)	+15%	-1%
Holst, 1959, India ⁴⁷	A	1354	0.66 (0.62-0.69)	0.98 (0.96-0.98)	0.67 (0.63-0.70)	0.98 (0.96-0.98)	+1%	0%
Kivihya-Ndugga (a), 2003, Kenya ⁴²	..	993	0.32 (0.27-0.35)	1.00 (0.99-1.00)	0.67 (0.62-0.70)	0.99 (0.97-0.99)	+35%	-1%
Kivihya-Ndugga (b), 2003, Kenya ⁴²	..	993	0.60 (0.55-0.64)	0.98 (0.96-0.99)	0.78 (0.73-0.81)	0.98 (0.96-0.99)	+18%	0%
Kumar, 1979, India ⁴⁸	AO	574	0.89 (0.85-0.92)	0.96 (0.93-0.98)	0.94 (0.90-0.96)	0.97 (0.94-0.98)	+5%	+1%
Narain (a), 1971, India ⁴⁹	..	436	0.84 (0.80-0.87)	..	0.90 (0.86-0.92)	..	+6%	..
Narain (b), 1971, India ⁴⁹	..	436	0.71 (0.66-0.75)	..	0.84 (0.80-0.87)	..	+13%	..
Singh (b), 1998, Nepal ⁴⁴	A	205	0.48 (0.38-0.56)	0.94 (0.86-0.98)	0.57 (0.47-0.65)	0.94 (0.86-0.98)	+9%	0%
Somlo, 1969, USA ⁴³	AR	3000	0.52 (0.46-0.58)	0.99 (0.98-0.99)	0.71 (0.65-0.76)	0.98 (0.97-0.98)	+19%	-1%
Tansuphasiri, 2002, Thailand ⁴⁵	AO	392	0.69 (0.59-0.77)	0.97 (0.94-0.99)	0.60 (0.50-0.68)	0.98 (0.95-0.99)	-9%	+1%
Truant, 1962, USA ⁴⁷	AR	585	0.78 (0.65-0.87)	..	0.93 (0.83-0.98)	..	+15%	..
Uluhanligil, 2000, Turkey ⁴⁸	AO	40	0.70 (0.53-0.83)	..	0.93 (0.79-0.98)	..	+23%	..
Weiser, 1966, USA ⁴⁸	AO	345	0.52 (0.37-0.65)	0.99 (0.97-0.99)	0.63 (0.48-0.75)	0.97 (0.94-0.98)	+11%	-2%

*See webtable for further details on studies. Difference between fluorescence microscopy and conventional microscopy (CM) sensitivity estimates: mean +10% (95% CI 5, 15). Difference between FM and CM specificity estimates: mean 0% (95% CI -0.9, +0.2). A=auramine; AO=auramine O; AR=auramine-rhodamine; ..=not reported.

Table 1: Studies comparing sensitivity and specificity of conventional and fluorescence microscopy

Steingart et al, Lancet Infectious Diseases 2006

Why a Reference Standard

- Accuracy of test
- Other “measurables”
 - Cost effectiveness
 - User friendliness
 - Time
 - Practicality

Limits of Detection



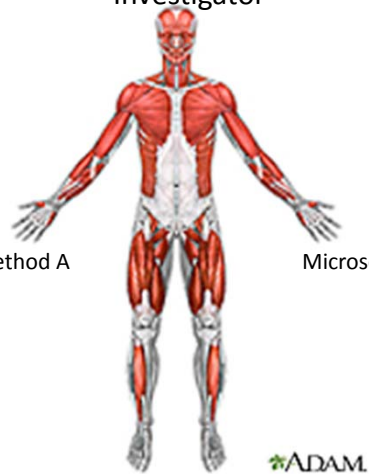

- Liquid culture: 10-100 cfu/mL
- Solid culture and automated NAAT: 50-150 cfu/mL
- Line probe: 1 000-10 000 cfu/mL
- Microscopy: 10 000 cfu/mL

Anatomy of a Microscopy study

Investigator

Microscopy method A

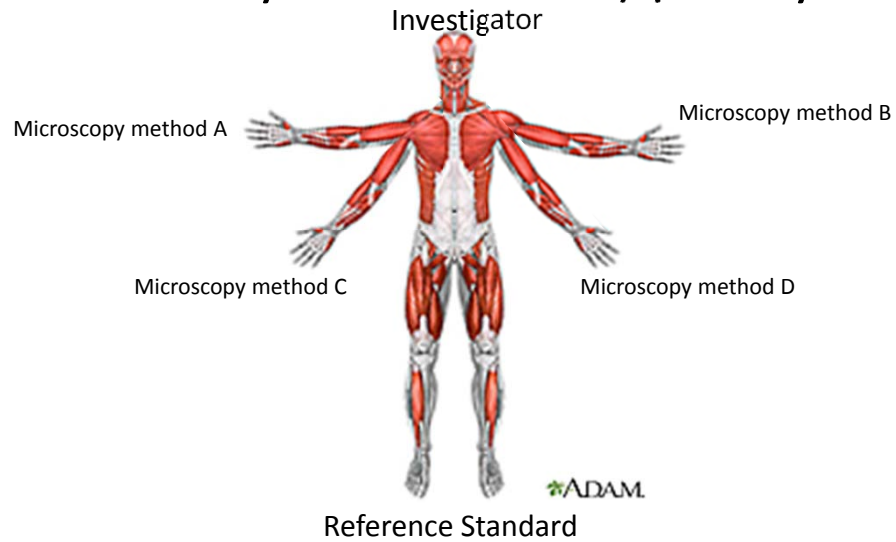
Microscopy method B



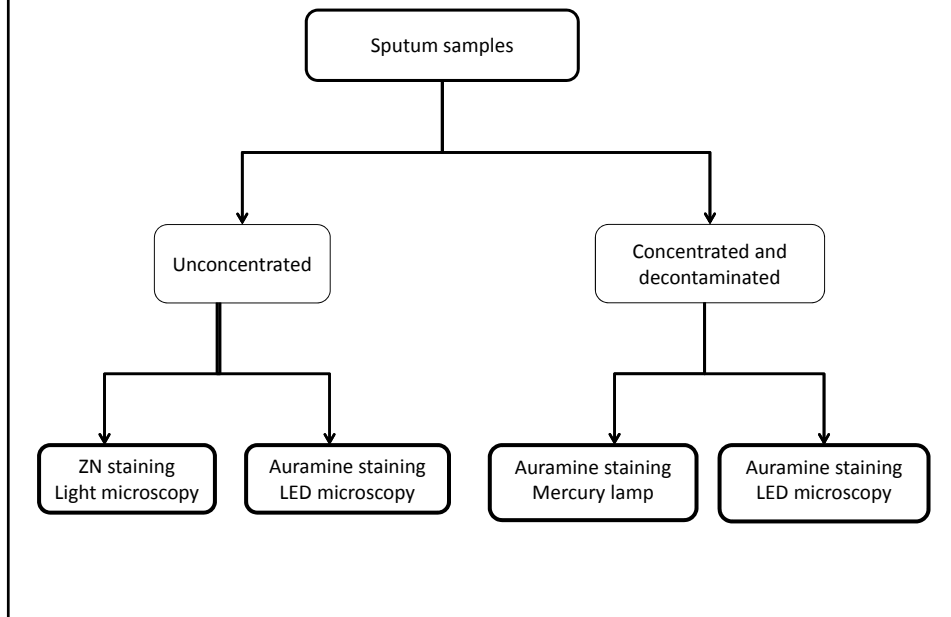
ADAM

Reference Standard

Anatomy of a Microscopy study



Study Design



Potential Reference Standards

- Microscopy
- NAATs
- Culture
- Clinical

Microscopy

- 4 different modalities
- Consider pos result in any sample equivalent to true pos

– Easy

	Direct ZN	Direct LED	Conc MVL	Conc LED	Any pos
No Pos	90	92	116	118	131
%	68,7	70,2	88,5	90,1	

- Assume no false positives
- Difficult to compare to other studies
- True accuracy unknown

Microscopy

- Blinded re-checking
- 2nd observer re-reads discordant slides

Table 2 Cross-tabulation of Fraen and LW LED systems crude results

Fraen	LW			Total <i>n</i>
	Negative <i>n</i>	Positive <i>n</i>	Scanty <i>n</i>	
Negative	1003	5	34	1042
Positive	50	680	49	779
Scanty	67	15	34	116
Total	1120	700	117	1937

LED = light-emitting diode.

Table 3 Evaluation of Fraen and LW LED systems, using microscopy positivity confirmed by rechecking in any system (*n* = 941) as the gold standard

System	True-positive <i>n</i>	False-positive <i>n</i>	True-negative <i>n</i>	False-negative <i>n</i>	Yield of true-positives % (95%CI)	False-positives % (95%CI)
Fraen	880	15	981	61	93.5 (91.9–95.1)	1.7 (0.9–2.8)
LW	806	11	985	135	85.6 (83.4–87.9)	1.4 (0.7–2.4)

LED = light-emitting diode; CI = confidence interval.

Affolabi, JTLD, 2010

NAATs

- Many available
- Automated
 - easiest, most reliable, most expensive
 - Some still in development - inappropriate as ref standard
- Non-automated commercial
 - Performance reasonable, expertise needed, cost
- In-house
 - Cheaper, more variable performance, expertise needed

Culture

- Still most sensitive / specific gold standard
- Not perfect
- Solid or liquid?
- How many?
- Expense, infrastructure
- Operator dependent

Table 1: 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% (1016/1092)	97.7% (1104/1130)	98.2% (551/561)
Smear-negative, Culture-positive	69.3% (205/296)	84.4% (276/327)	72.5% (124/171)
All Culture-positive	88.0% (1221/1388)	94.7% (1380/1457)	92.2% (675/732)

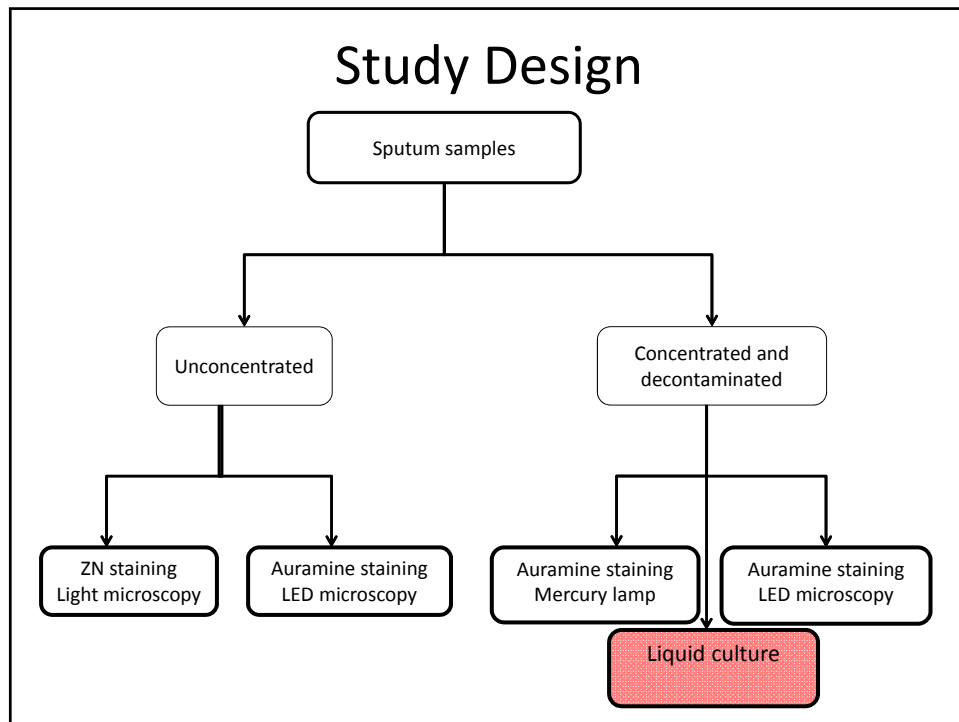
* Calculated as the proportion of individual cultures that agrees with the reference standard of 3 smears and 4 cultures. Contaminated cultures are excluded. Patients with two valid cultures appear in the calculation twice.

† Results are shown for the direct MTB/RIF test performed on unprocessed sputum. For details, see manuscript, table 2.

Boehme, NEJM 2010, online suppl

Clinical

- Not used in isolation
- Composite with culture / microscopy / NAAT etc
 - Definite
 - Possible / probable
 - Not TB
- Potentially more robust
- More complex, challenging
- Out of realm of pure laboratory assessment



Other Good Intentions

- Different readers for each smear
 - Difficult to maintain in routine lab
 - Experience of reader important
- Blinding
 - To culture – relatively easy
 - To other smears – more difficult
- Reading different smears as opposed to re-staining / re-reading same smear
- Rechecking of inconsistencies
 - Realistic?

LED Study Results

- 649 samples, single MGIT culture
- Exclude contaminated cultures (28) and NTMs (5)

TABLE 1 Sensitivities, specificities, and positive and negative predictive values for each of the four different smear detection methods stratified by HIV status and CD4 cell count

Study group	Sputum samples n	Sensitivity				Specificity			
		Unconcentrated sputum sample		Concentrated sputum pellet		Unconcentrated sputum sample		Concentrated sputum pellet	
		ZN	LED	MVFM	LED	ZN	LED	MVFM	LED
All	616	49 ^{†,§} (42–57)	52 ^{†,‡} (44–59)	66 (58–73)	66 ^{§,†} (58–73)	99 (98–100)	100 (98–100)	99 (98–100)	99 (98–100)
HIV-negative	377	55 [§] (45–65)	57 [†] (46–66)	74 ^{†,§} (64–82)	71 ^{††} (61–79)	99 (97–100)	99 (97–100)	99 (97–100)	99 (97–100)
HIV-positive	150	39 ^{†,††,§§} (26–54)	46 ^{†,††} (32–61)	46 ^{†,§,} (32–61)	54 ^{†,§,§§,} (39–68)	100 (97–100)	100 (97–100)	100 (97–100)	99 (95–100)
CD4 count <200 cells mL⁻¹	69	39 (20–61)	50 (29–71)	44 ^{†,§,§§} (25–66)	56 ^{†,§,§§} (34–75)	100 (93–100)	100 (93–100)	100 (93–100)	100 (93–100)

Culture positivity to *Mycobacterium tuberculosis* served as the reference standard. Data are presented as median (95% CI), unless otherwise stated. ZN: Ziehl-Neelsen staining light microscopy; LED: auramine O staining light-emitting diode fluorescent microscopy; MVFM: auramine O staining conventional mercury vapour fluorescent microscopy. [†]: p=0.33; [‡]: p=0.001; [§]: p=0.005; ^{||}: p=0.08; ^{§§}: p=0.28; ^{††}: p=0.002; ^{§§§}: p=0.06; ^{†††}: p=0.25; ^{§§§}: p=0.002; ^{|||}: p=0.5; ^{††††}: p=0.5.

Whitelaw, Peter ERJ 2011

Hierarchy (for microscopy)

- Culture
- NAAT (preferably commercial, automated)
- Blind rechecking of microscopy
- Any positive microscopy

