



Reference Standards for Serological Diagnostic Tests for TB

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Reference Standard for Serological Tests

A test that is currently accepted as a reasonably, but not necessarily, as 100% accurate. It is used as the reference method (gold standard) for assessing performance of other test methods.

Culture of *M. tuberculosis* is the gold standard for TB diagnosis

A serological assay should NOT usually be compared to an assay that detects the micro-organism directly.

How will using cultures as gold standard for SD test (immunological assay) work?

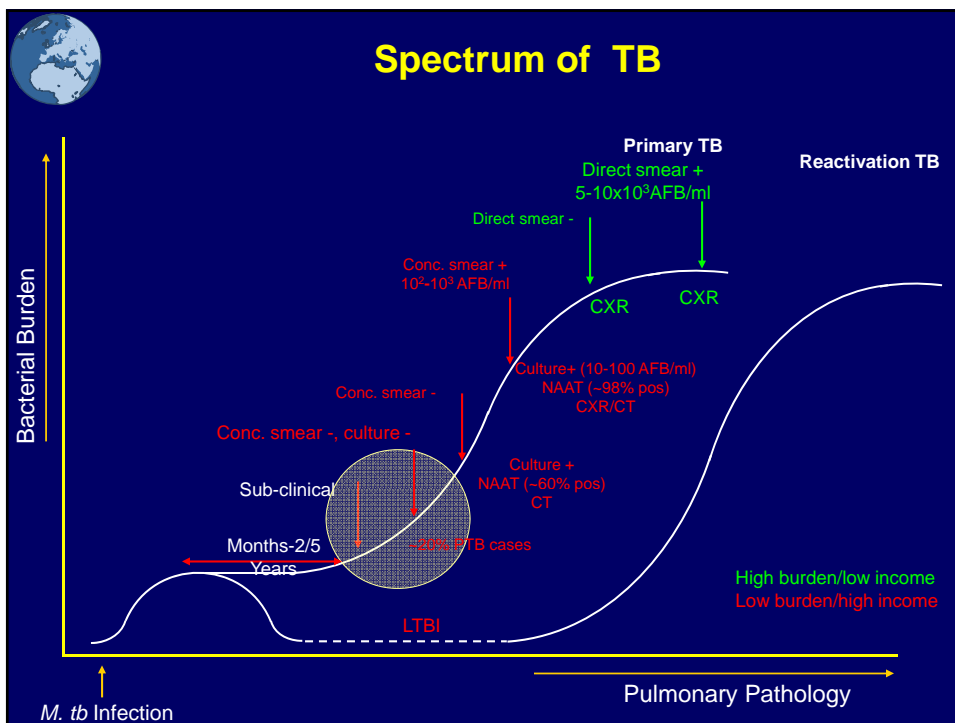


Specifications for a Rapid POC Test

- **Sensitivity:**
 - >95% for smear positive, culture positive patients
 - ~60-80% for smear negative, culture positive patients
- **Specificity:**
 - >95% compared to culture

Comparable to the performance of NAAT
NAAT was FDA approved in 1995/1999
 No NAA POC test yet available for any disease

Even with culture, clinical judgement finally rules





Sub-clinical TB in HIV- High Risk Subjects

•A subset of asymptomatic close contacts of infectious TB patients progress to symptomatic TB within months of follow up. This asymptomatic subset must have active subclinical infection.

Canada: In 6 months of follow up, 6% of adult contacts of smear-positive TB cases developed TB whereas <1 of adult contacts of smear negative TB cases developed TB. (Grzybowski et al.1975: *Bull. Int. Union. Tuberc* 50 (1): 90)

Malawi: Seven percent of the PTB patients had HH members who were diagnosed with TB in the previous 12 months, 1% of controls had TB in the family during the same time period. (Claessens et al. 2002: *Int. J. Tubercle. Lung Dis* 6 (3): 266)

•Immunological changes are detected during the months prior to manifestation of TB in close contacts who progressed to TB.

Pakistan: Six percent of HHC developed TB in 2 years of follow-up. Sharp decline in γ IFN production prior to development of TB. (Hussain et al. 2007: *Clin. Vaccine Immunol.* 14 (12):1578)

Portugal: 6/10 HCW who cared for TB patients developed TB in 5 years of follow-up; no HCW with no contact with TB patients developed TB. Increased TH2 responses during months prior to clinical TB. (Ordway et al. 2004: *JID.* 190:756-766).

Ethiopia: 29% of high responders progressed to TB??



Sub-clinical TB in HIV+ Subjects

- Active TB estimated to be present upto 1.3 years prior to diagnosis in HIV+ and upto 4.2 years in HIV- African Gold Miners.

Corbett EL et al *Am J Respir Crit Care Med* 170:673, 2004

- Intensified case finding studies have shown that ~ one fifth of HIV+ patients from Africa and Asia who have culture proven TB are asymptomatic (across a range of CD4 T cell counts)

Kranzer K et al., *Lancet Infect Dis* 10, 93, 2010
Mtei L et al., *Clin Infect Dis* 40, 1500, 2005

- Antibodies to MS, MPT51, PPE55 present in retrospective sera obtained upto 6 months before TB diagnosis in HIV+ patients from the US and India

Laal S et al., *J. Infect. Dis.* 176:133, 1997
Singh KK et al., *Infect Immun* 73:5004, 2005
Singh KK et al., *Clin Diagn Lab Immunol* 12:354, 2005
Wanchu et al., *PLoS One* 3: e2071, 2008

- Antibodies to MS, ESAT6, CFP10 present in retrospective sera obtained from HIV co-infected individuals who developed active TB during a multicenter prospective study on pulmonary complications of HIV/AIDS conducted among >1300 subjects in the USA in the 1980s.

- Abs were present upto 20 months before manifestation of TB

Gennaro ML et al., *Int J Tuberc Lung Dis* 11:624, 2007



Ruling out TB

- Response to short course of broad spectrum antibiotics plays a major role in the clinical decision of ruling out TB.
- Studies from different settings have shown that between 8-50% of the SN TB patients with abnormal chest X rays who ultimately had positive *M. tb* cultures showed symptomatic response to a trial of antibiotics
 - Wilkinson et al 1997. *Trans R Soc Trop Med Hyg*: 91: 422-424)
 - Somi et al 1999. *Int. J Tuberc Lung Dis*: 3: 231-238).
 - Oyewo et al: 2001; *Int. J Tuberc Lung Dis*: suppl 1; S216).
 - Bah et al 2002. *Int. J Tuberc Lung Dis*: 6: 592-598)
- Symptomatic or clinical response to broad spectrum antibiotics has poor discriminatory power and does not reliably exclude TB.



Potential Targets for a Rapid Test

Steingart et al, 2009. Performance of Purified Antigens for Serodiagnosis of Pulmonary Tuberculosis: a Meta-analysis. *Clin Vaccine Immunol* 16:260-276 .

Malate Synthase (GlcB or *M.tb* 81)

TBF6+DPEP

73-75% sensitivity in smear positive TB (case control studies)

Combinations of select antigens provide higher sensitivity



Specificity of anti-MS Abs

TABLE 8. Specificity estimates by type of comparison

Antigen name	Specificity (%) ^a	
	Patients with nontuberculous respiratory disease	Healthy subjects
Recombinant 38 kDa	97 (90–99) (6)	90 (57–99) (6)
Recombinant malate synthase	97 (91–100) (4)	99 (81–100) (4)
Recombinant CFP-10	99 (92–100) (3)	90 (43–99) (3)
Native 38 kDa	96 (90–99) (6)	98 (92–100) (4)
DAT	55 (30–76) (4)	97 (88–100) (3)

^a The data represent the posterior means (95% credible intervals) (number of studies).

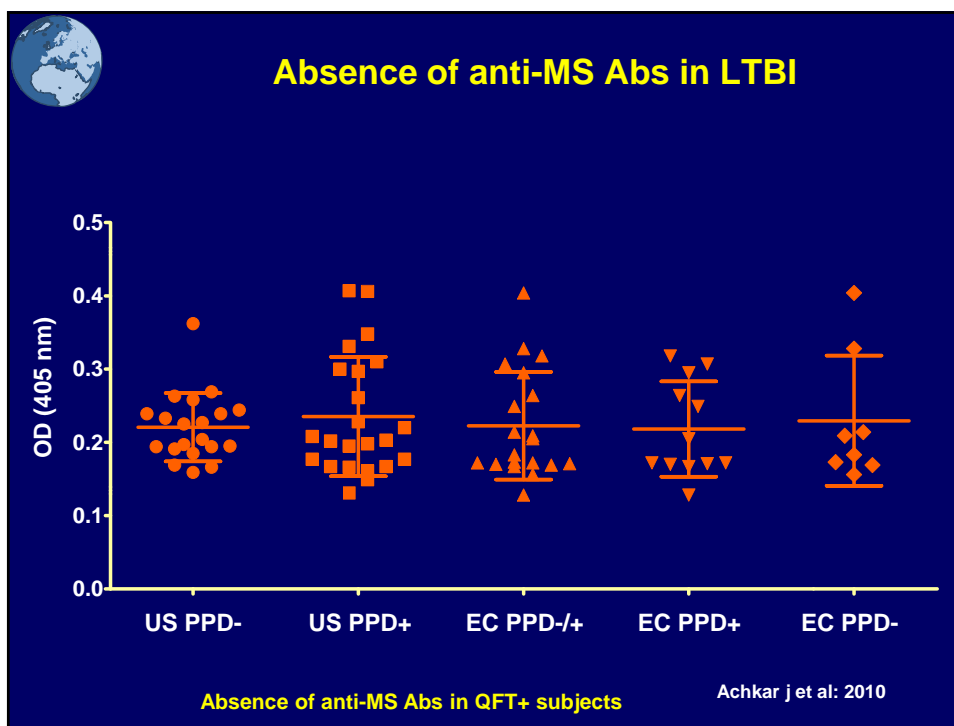


Specificity of Anti-MS Abs

Reference	Protein	Sensitivity %		Specificity %*
		HIV-TB+	HIV-TB-	
Hendrickson, et. al (2000)	MS	57 (n=67)	92 (n=27)	98 (n=97)
Houghton, et. al (2002)	MS	58 (n=66)	80 (n=64)	97 (n=141)
Mukerjee, et. al. (2004)	MS	44 (n=83)	87 (n=47)	97 (n=54)
Singh, et. al. ** (2005)	MS	70 (n=40)	79 (n=24)	98 (n=59)
Wanchu, et. al. *** (2008.)	MS	75 (n=138)	78 (n=60)	97 (n=90)

Specificity tested in PPD+, PPD- subjects from SE Asia, Latin America, Russia, China Patients with Pneumonia, Asthma, NTM, HIV+ subjects etc.

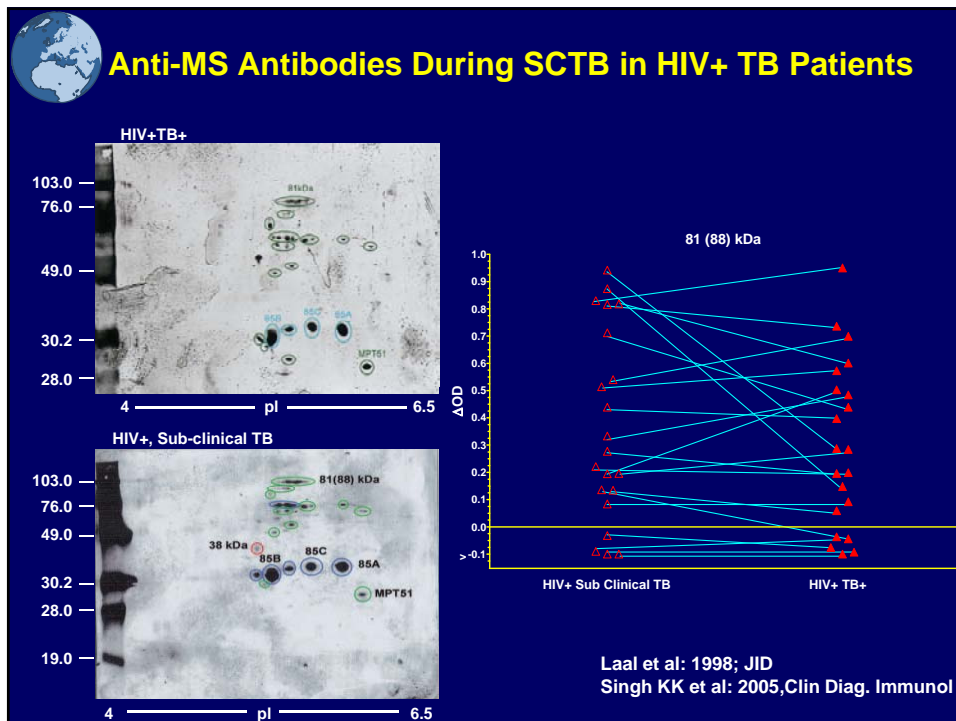
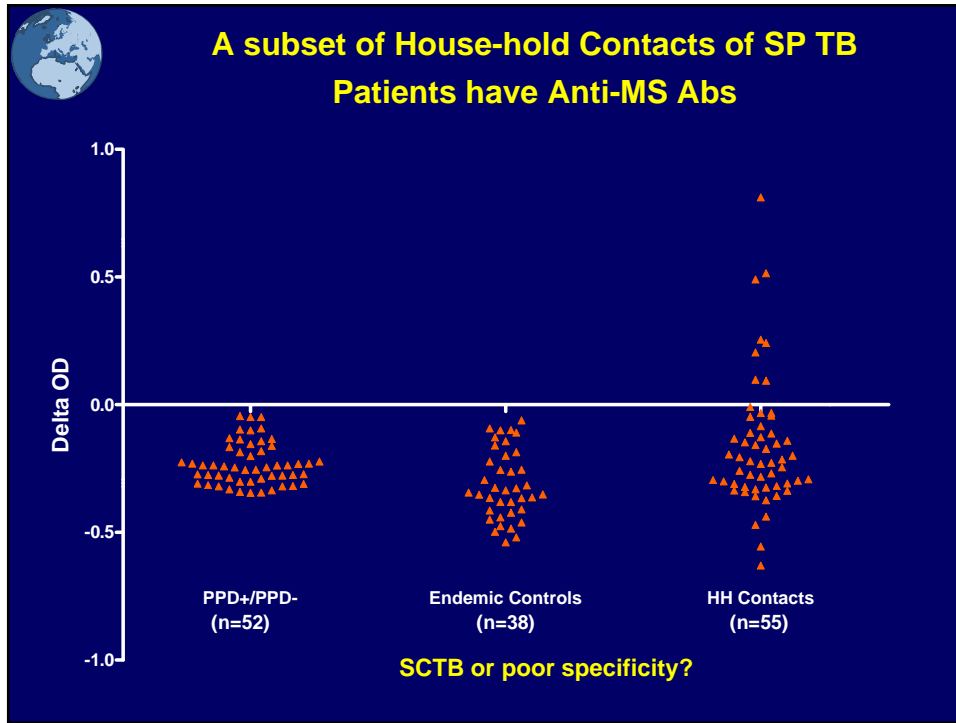
No difference in anti-MS responses between subjects with no LTBI, recent LTBI and previous LTBI (Rabahi M.F. et al., 2007. *BMC Infect Dis*, 7: 148)

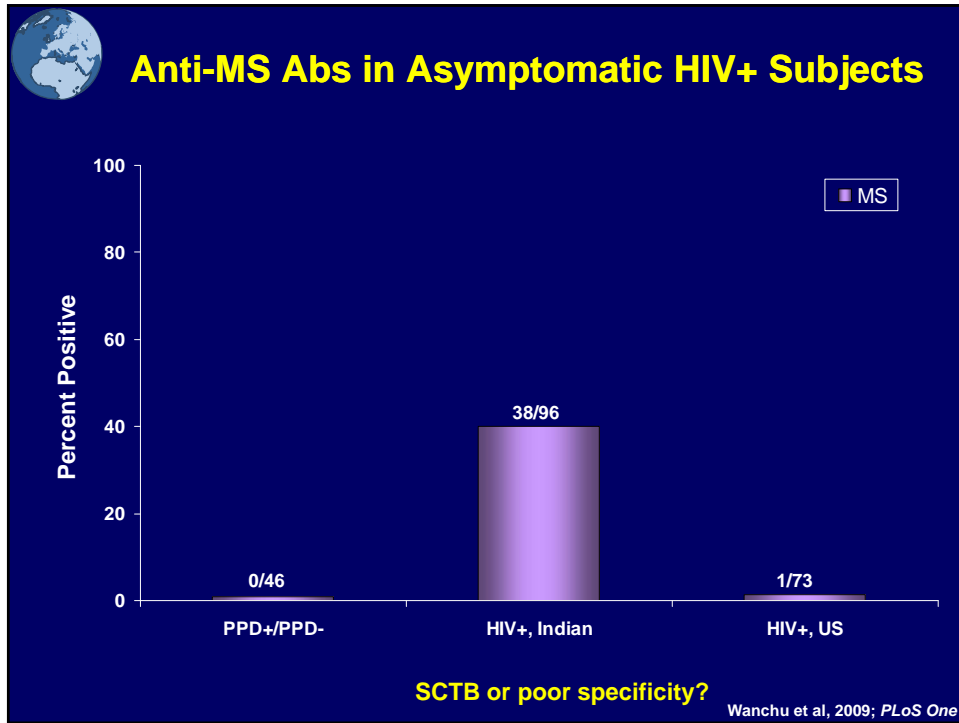


Absence of anti-MS Abs in Patients with NTBLD & NTBLI

Patient Status ^a	Source	n	Smear	No. (%) positive		
				TbF6 + DPEP	Mtb81	TbF6 + DPEP + Mtb81
HIV+TB+	Sub-Saharan Africa ^c	59	+	29	46	49
		5	-	1	5	5
	Total	64		30 (46.9)	51 (79.7)	54 (84.4)
HIV-TB+	Sub-Saharan Africa	66	+	47 (71.2)	38 (57.6)	56 (84.8)
HIV+TB-	United States	11		0	0	0
PPD+ ^b	Africa-Europe-Asia-Americas	57		3	2	4
PPD- ^b	Africa-Europe-Asia-Americas	29		1	0	1
Lung Cancer	China	13		0	0	0
Bone Cancer	China	4		0	0	0
Non-TB lung infections	China/Caucasian	18		0	1	1
Healthy	China/Caucasian	9		0	0	0

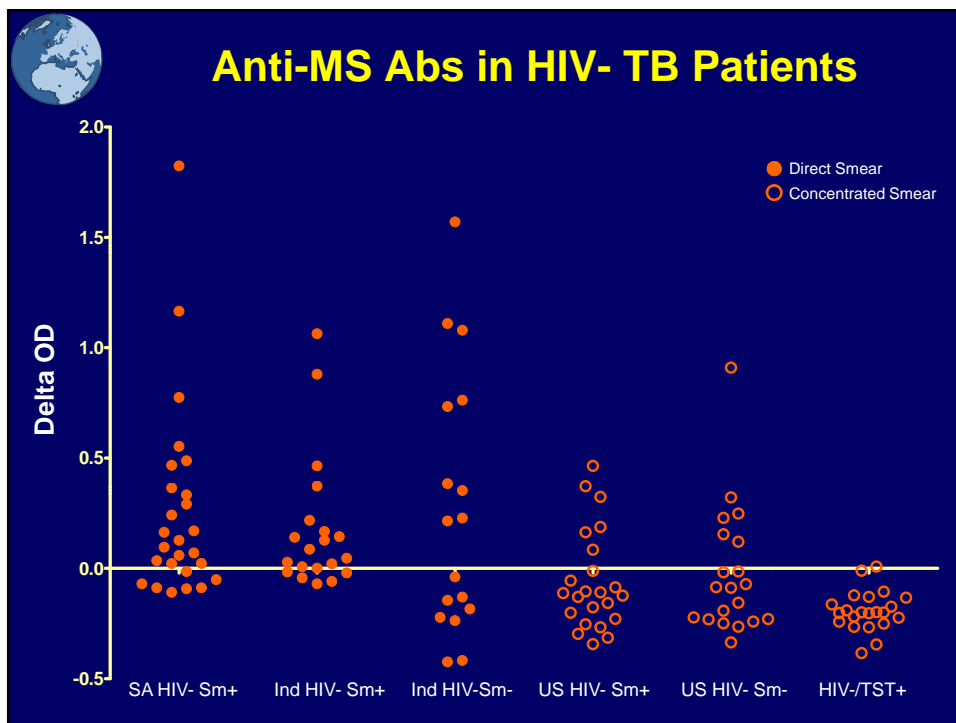
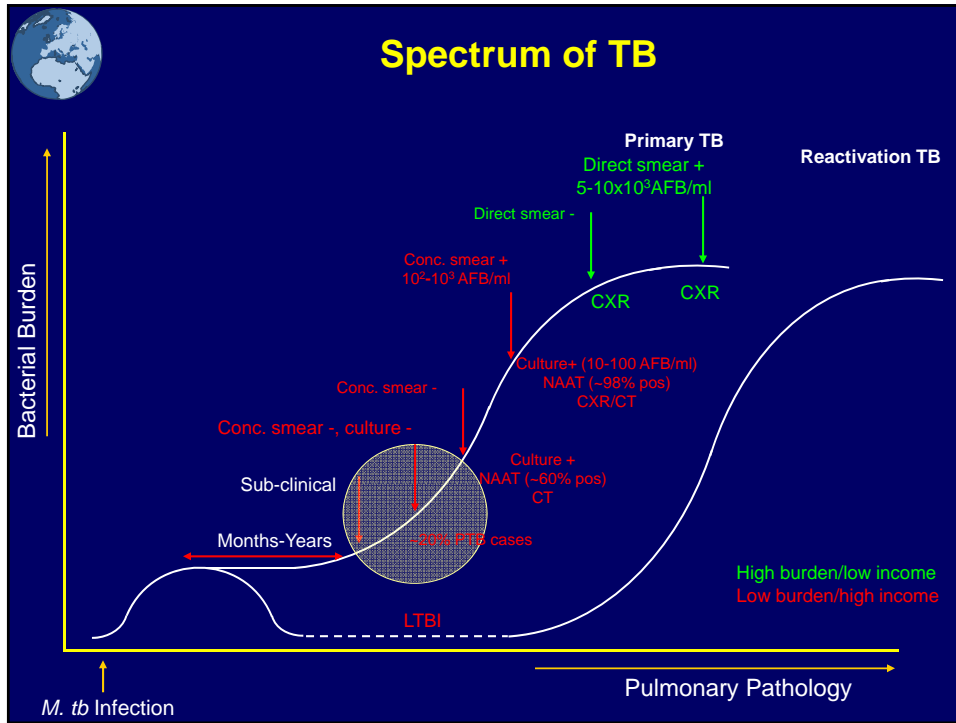
Houghton RL, et.al. (2002) *Clin Diagn Lab Immunol*





Culture as reference standard for a SD?

- The immune system can respond to *in vivo* antigen at thresholds that are lower than are detected by culture.
- Does the presence of anti-MS antibodies indicate a high-risk for progression to TB or poor specificity?
- Studies with well characterized, long term followed cohorts of high-risk individuals may provide answers.





Sensitivity of a SD Test

What must the SD be as sensitive as?

Direct sputum smear?
 Concentrated sputum smear ?
 NAAT
 Solid culture, Liquid culture

“The sensitivity of the direct sputum smear is highly variable. Even a standardized POC test that can replace the direct smear will revolutionize TB control”.

(Max Salfinger)



Reference Standards for SD

Sensitivity: Culture (but rethink sensitivity of POC)

Specificity:

- Endemic subjects
- PPD+/IGRA+ subjects
- BCG Vaccinated subjects
- NTBLD Patients
- NTBLI Patients
- HIV+TB- Patients at low risk for TB
- Culture negative TB suspects