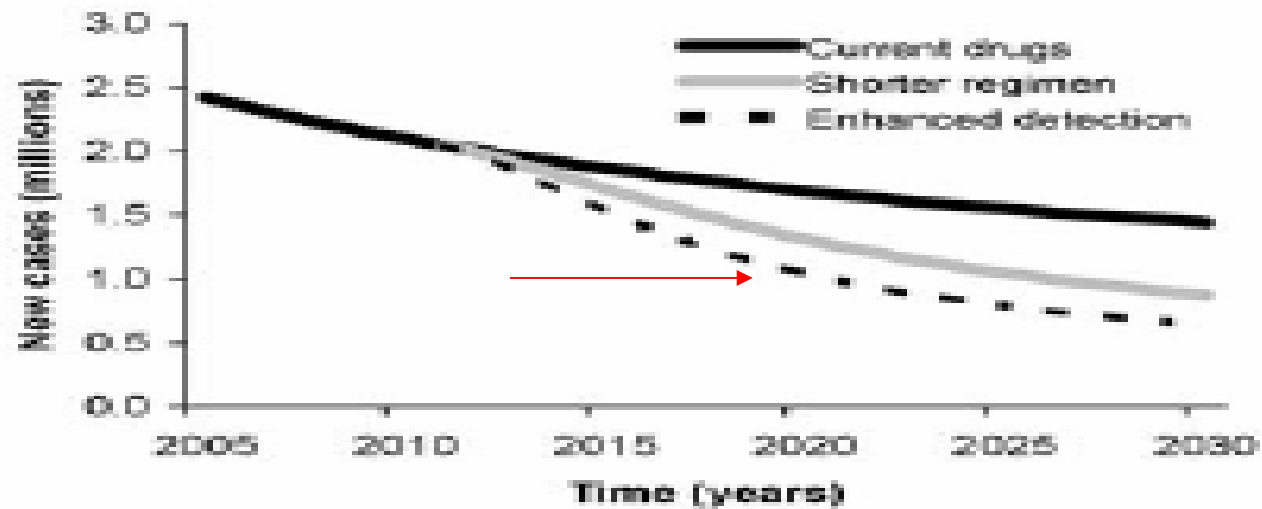


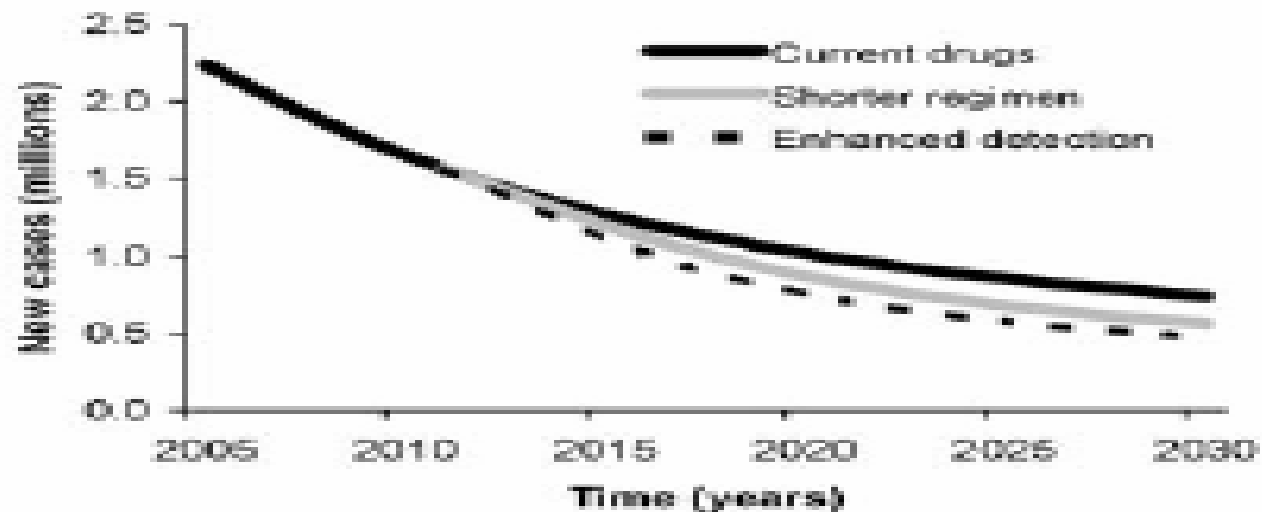
**Option and practical
application for fluorescence
microscopy in low income
countries**

**Prof Richard Urbanczik M.D.
Germany**

A. Stable-DOTS baseline: constant 42% case detection



B. DOTS-target baseline: 70% case detection by 2009



Projected trends of new TB in SE Asia; SALOMON JA et al, Prospects for advancing TB control efforts through novel therapies, PLoS Med 2006, 3(8): e273 DOI: 10.1371/journal.pmed.0030273

MUGUSI F et al, HIV co-infection, CD4 counts & clinical correlates of bacillary density in PTB, IJTLD 2006,10: 663

	N°	% with high AFB density ¹	Adjusted prevalence ratio	Adj. p
HIV-negative	411	<u>75.7</u>	1.0	
HIV-positive	433	<u>39.0</u>	0.54 (0.45 – 0.59)	<0.001

¹ Setting in Dar Es Salaam, high AFB density = > 10 AFB / field, Z-N stain

STEINGART KR, HENRY M, NG V, HOPEWELL PhC, RAMSAY A,
CUNNINGHAM J, URBANCZIK R, PERKINS M, AZIZ M, PAI M,
**Fluorescence vs. conventional sputum smear
microscopy for TB: a systematic review,**
Lancet Inf Dis 2006. 6:570 - 81

- **FM was on average 10 % more sensitive than CM – median 11 %, range – 9% to + 35%.**
- On average, specificity of FM was comparable to that of CM – median 0 %, range – 2% to + 1%.
- **FM reading was significantly shorter than CM.**

CM = conventional microscopy, FM = fluorescence

F.BA & H.L.RIEDER, Int J Tuberc Lung Dis
3:1999, 1101

Fluorescence

ZN

	Neg	1-3	4-9	+	++	+++
Neg	1165	35	3	1	0	0
1-3	4	10	1	2	1	0
4-9	1	2	4	6	0	0
+	2	3	3	34	6	1
++	0	1	1	8	43	7
+++	0	0	0	2	23	122

Improving results of FM

- Improving specimen quality
 - Facilitating the collection & transportation of specimens
 - Homogenization & concentration
 - Improving staining for FM
-
- Changing FM technology (microscope)
 - EQA for FM stained smears

**Better patient instruction for sputum sampling can improve microscopic TB diagnosis,
ALISJAHBANA B et al, Int J Tuberc Lung Dis 2005, 9: 814 – 7
[Setting in Indonesia]**

	Control n = 93	Interven. n = 81
Patients		
- ZN positive	35 %	51 %
- 1st sputum positive	26 %	46 %
- 2nd sputum positive	31 %	40 %
- 3rd sputum positive	27 %	41 %
Sputum samples		
- more than 5 ml	2.2 %	23.2 %
„scanties“ (+)	16.0 %	12.7 %



Sputum collection rooming, Thailand near Phuket, 2004

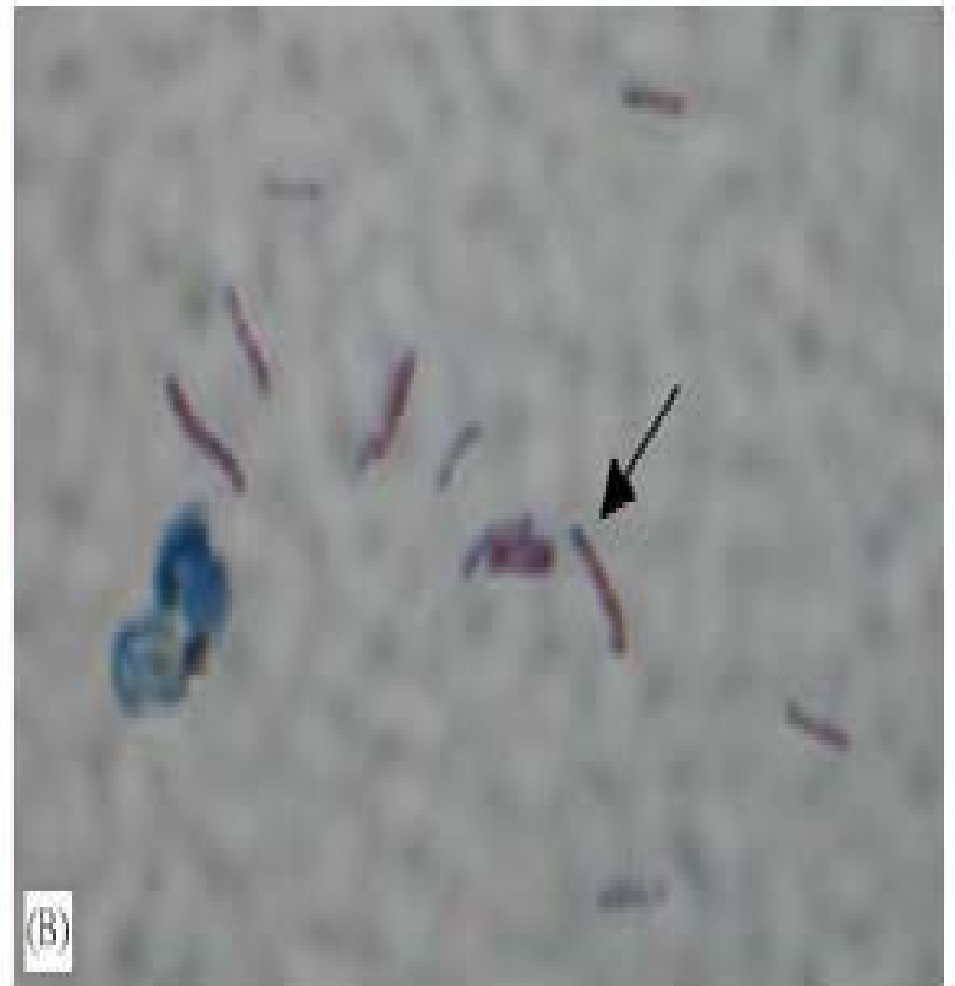


Fig. 1 Direct (A) and bleach digested (B) hot ZN smears of the same sputum specimen. The arrows point to acid fast bacilli.

LAWSON L, YASSIN MA, RAMSAY A et al, Microbiological validation of smear microscopy after sputum digestion with bleach, Tuberculosis 2006, 86: 34 - 40

LAWSON L, YASSIN MA, RAMSAY A et al,
Microbiological validation of smear microscopy after
sputum digestion with bleach; a step closer to a one-
step diagnosis of PTB, Tuberculosis 2006, 86: 34-40

- The conventional approach, using the spot – early morning – spot scheme **identified 51% of the 455 culture positive PTB cases.**
- In comparison, a single bleach digested smear **identified 50% of these cases**

-
- YAZDANI A et al, Sputum induction by oral salbutamol, Ind J Tuberc 2002, 49: 221
BALASUBRAMANIAM R et al, ,
Ind J Tuberc 2004, 51: 191-8

McCARTER Y.C. & ROBINSON A, Detection of AFB in concentrated primary specimens smears stained with rhodamine-auramine at room temperature and 37°C, J Clin Microbiol 1994, 32: 2487-9

- **Total of 782 specimens (70.5% of them respiratory, sputum & BAL) stained at both temperatures and evaluated blindly.**
- **30 specimens positive for AFB by both methods, additional 5 only at 37°C, additional zero at room temperature only.**

-
- ✓ **SETHI S et al, Indian J Pathol Microbiol 2003, 46: 521-3 = 300 sputa, 58 M+ by both temperatures, additional 5 at 37°C only, zero at room temperature.**

Possible advantages of FM used on a large scale

- Considerable decrease of workload.
- Sensitivity is higher than with CM, **particularly with low number of AFB**
- Specificity appears to be \approx similar to that of CM (for *M.tuberculosis*-complex)
- Data on preferential use in TB/HIV are equivocal, operational research needed (stage of HIV/AIDS ?)

Possible problems with FM used on a large scale

- Feasibility of FM in low income countries (electricity, limited human resources & inadequate training). **Multipurpose microscope is a must in the periphery.**
- Lack of internationally agreed EQA for FM
- Uncertainty about the stability of FM reagents under field (tropical) conditions
- Implementation is therefore likely to be complex, need of operational research.