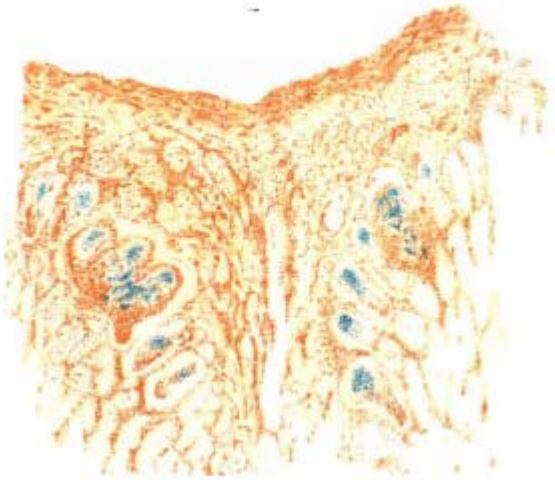
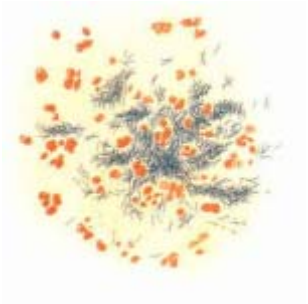
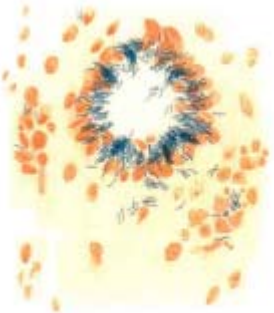




Microscopy

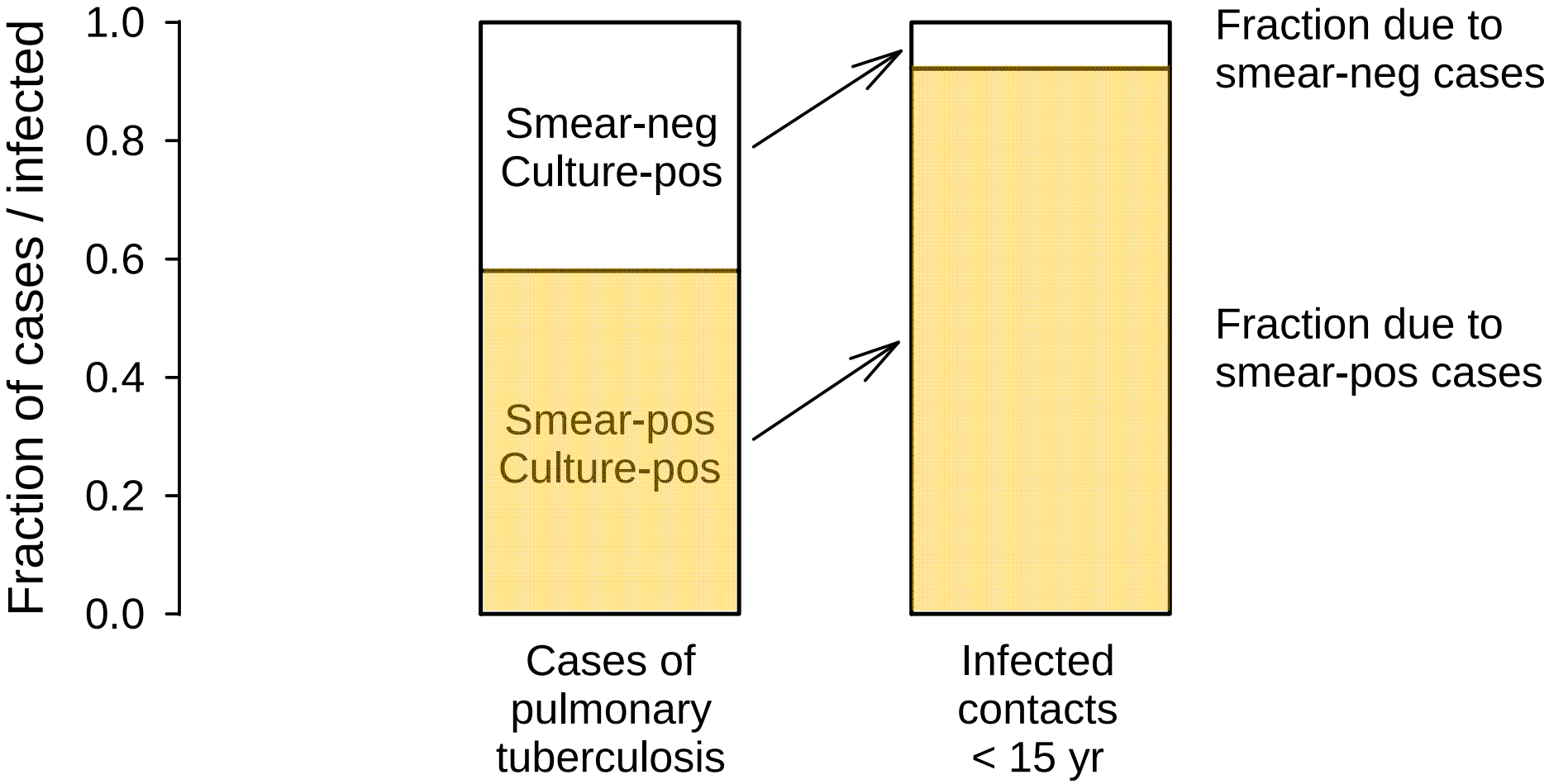
Münchenwiler, March 25, 2010

Hans L Rieder



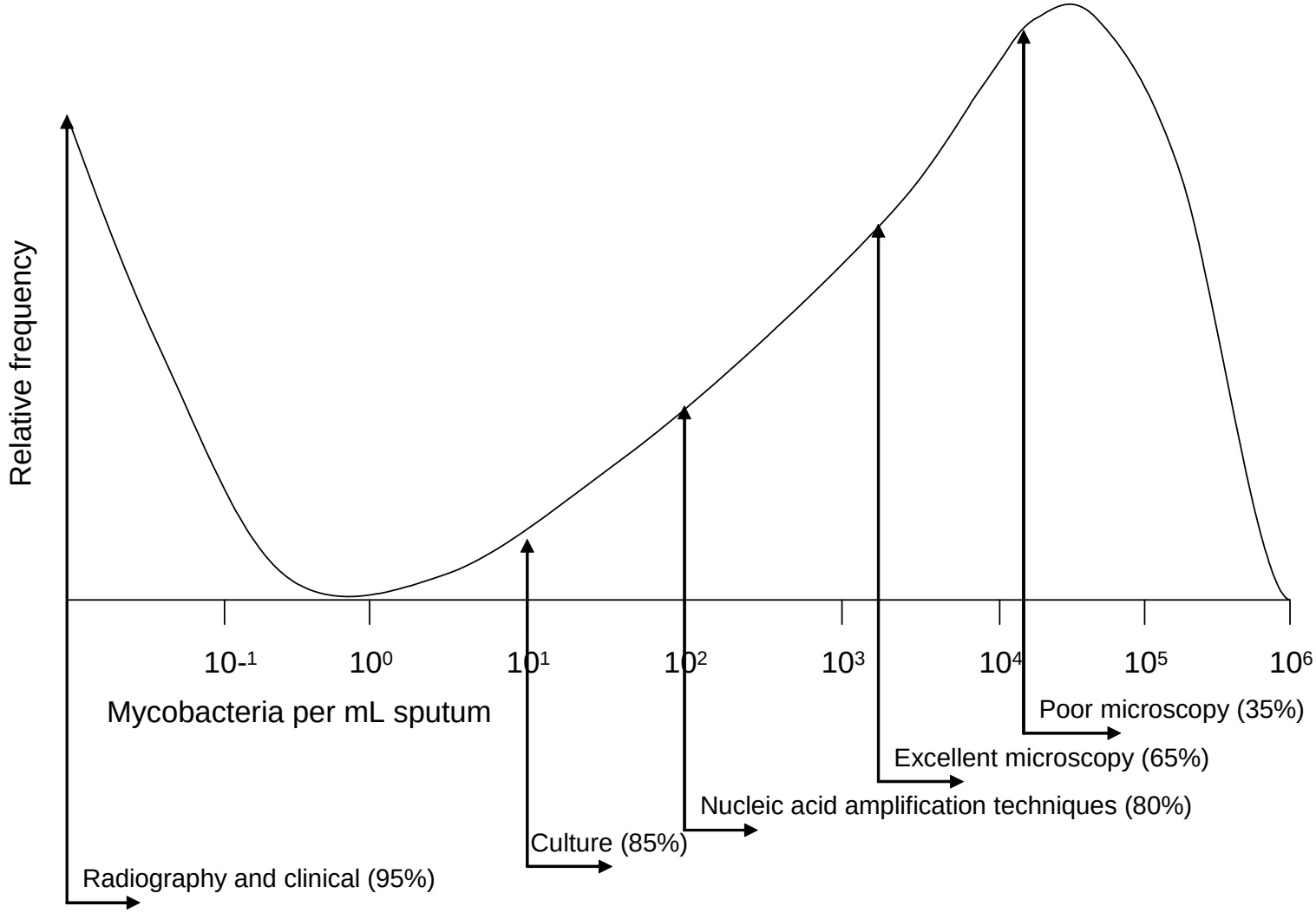
Picture drawings: Koch R. Mittheil Kaiserl Gesundheitsamt 1884;2:1-88

Sensitivity of Direct Sputum Smear Examination in Identifying Pulmonary Tuberculosis and Transmitters



Calculated from data from:
Grzybowski S, et al. Bull Int Union Tuberc Lung Dis 1975;50:90-106

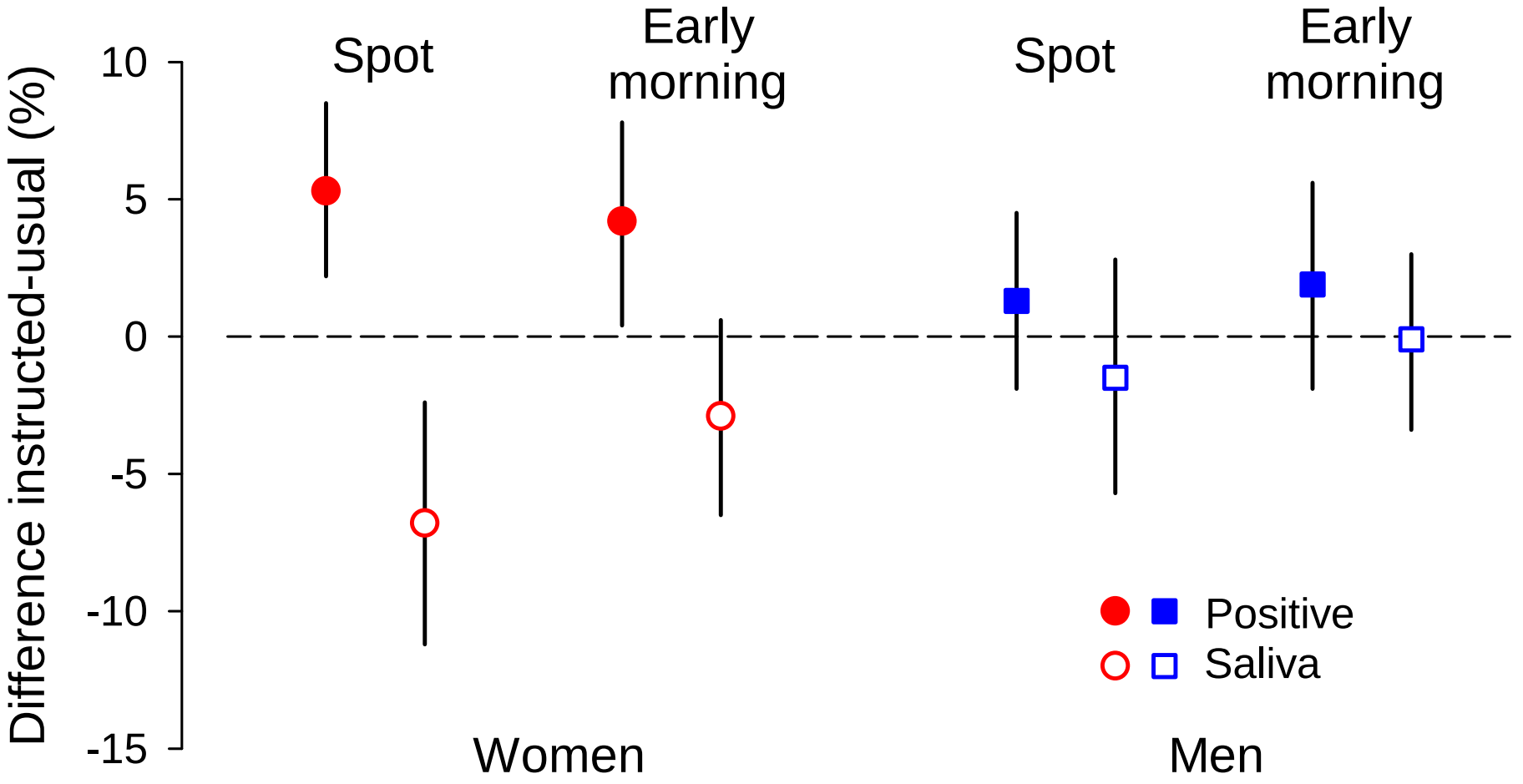
Schematic Presentation of Relative Frequency of Patients, Number of bacilli, and Available Diagnostic Methods



Rieder H L, Van Deun A, Kam K M, Kim S J, Chonde T M, Trébucq A, Urbanczik R. Priorities for tuberculosis bacteriology services in low-income countries. Second edition. Paris: International Union Against Tuberculosis and Lung Disease, 2007

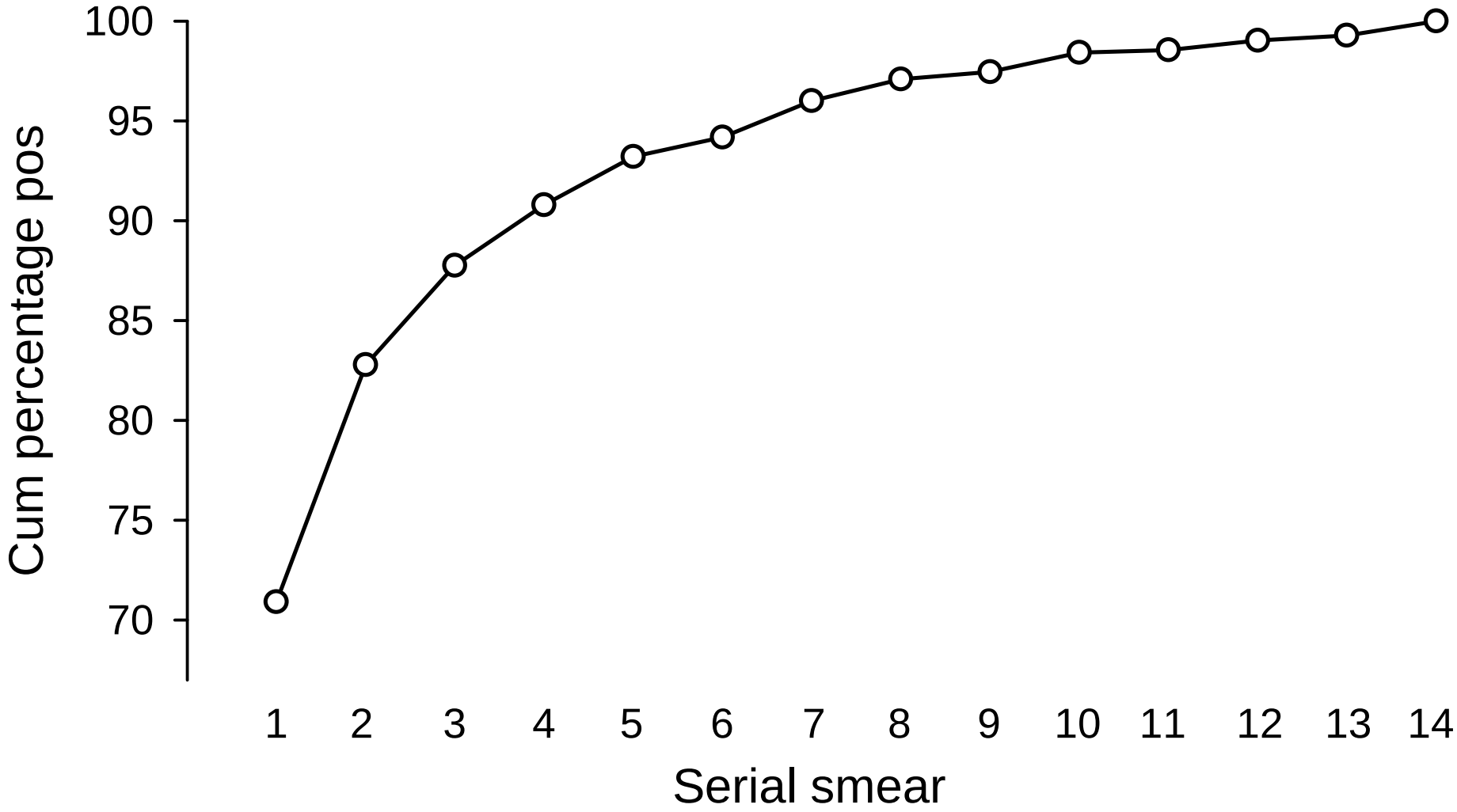
Where things may not be optimum and adversely impact on sensitivity of the sputum smear microscopy result	
Step	Problem area
Sputum production	Patient instruction on what constitutes a good sputum sample
Sputum processing	Non-homogeneity of sputum, smear preparation
Staining	Quality of fuchsin
Examination	Time spent on examination, actual number of fields properly examined

Improving yield of sputum smear microscopy by simple sputum-submission instructions, Pakistan, 2005



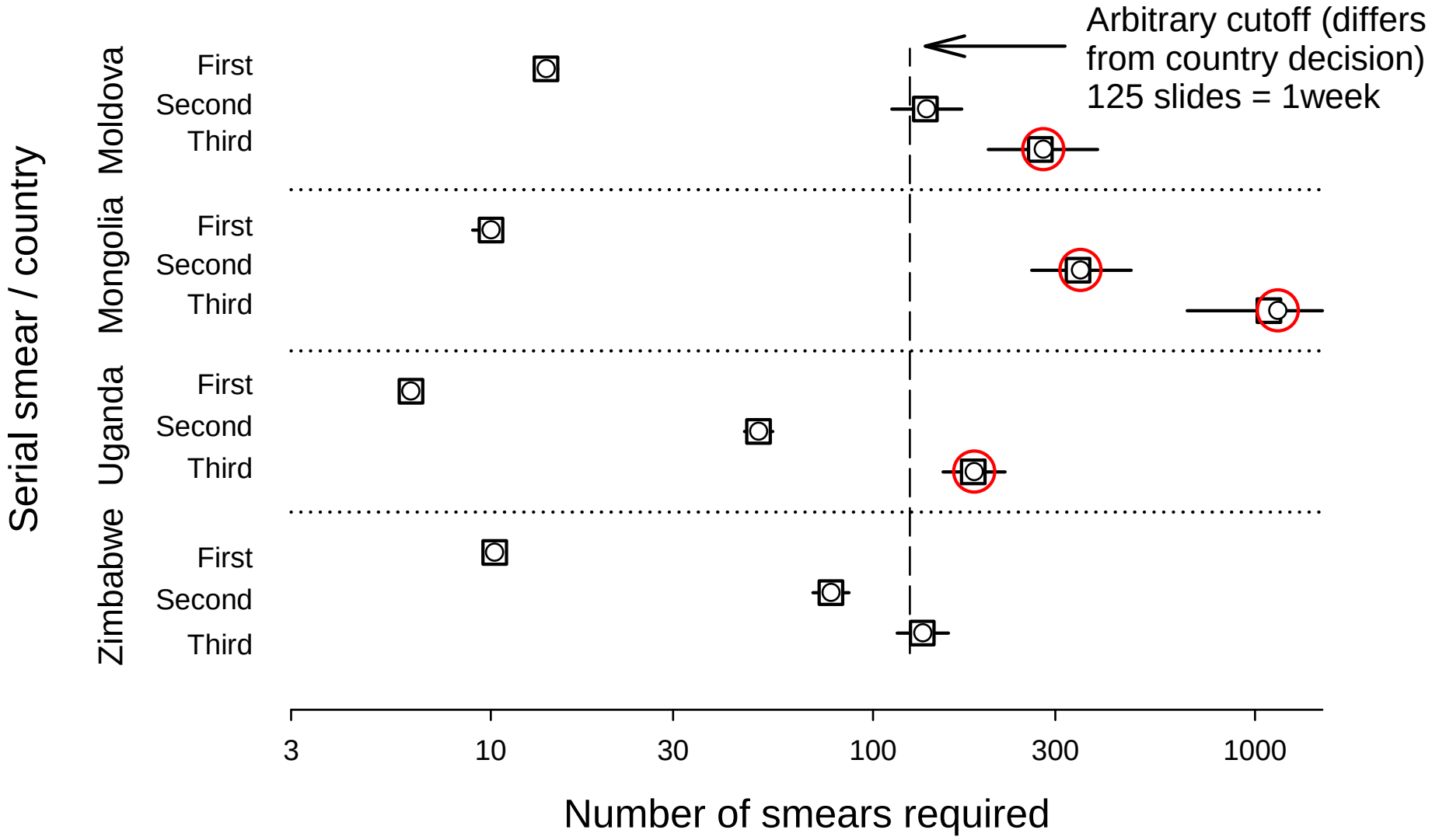
Sameer Khan M, et al. Lancet 2007;369:1955-60

Cumulative Yield of Sputum Smear Examination Among 825 Smear-Positive Patients, United Kingdom, 1930s

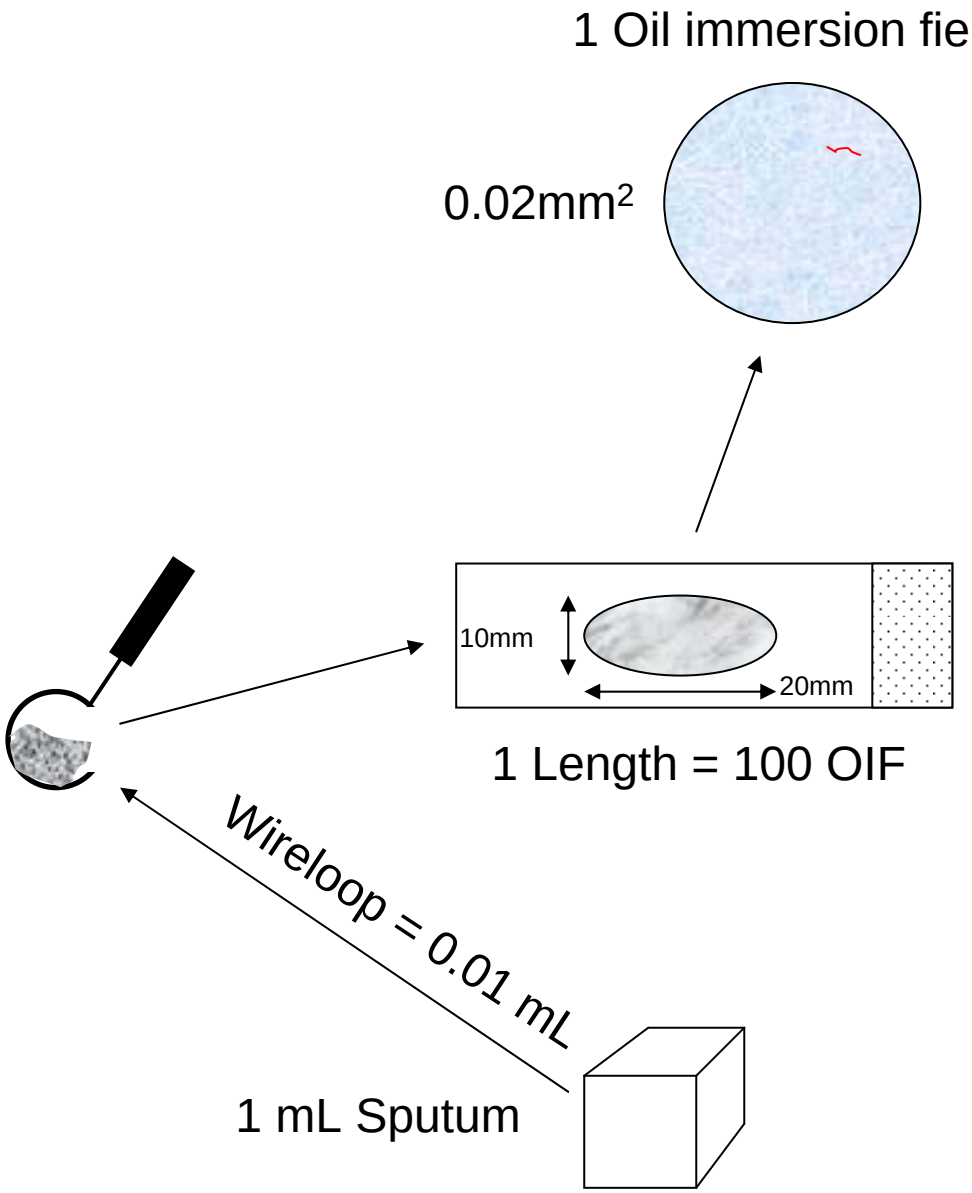


Hunter RA. Tubercle 1940;21:339-59

Number of Examinations Required for Successive Serial Smears to Identify one Additional Case, by Country



Mabaera B, et al. Int J Tuberc Lung Dis 2006;10:1030-5
Katamba A, et al. Int J Tuberc Lung Dis 2007;11:659-64



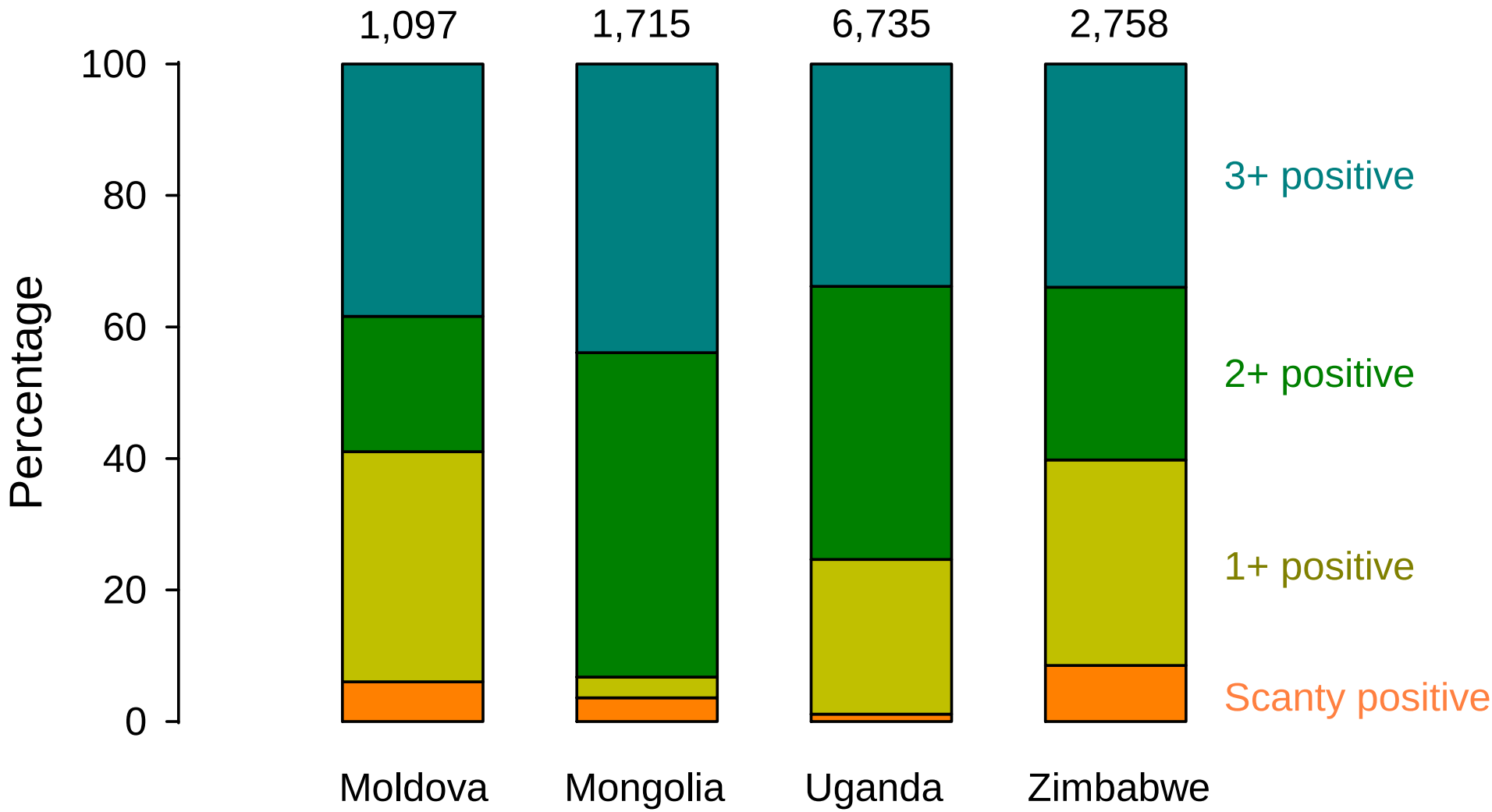
To see:
1 AFB in 100 fields

requires:
smear surface of 200 mm²
containing 100 AFB

which requires:
1 mL sputum
containing 10,000 AFB

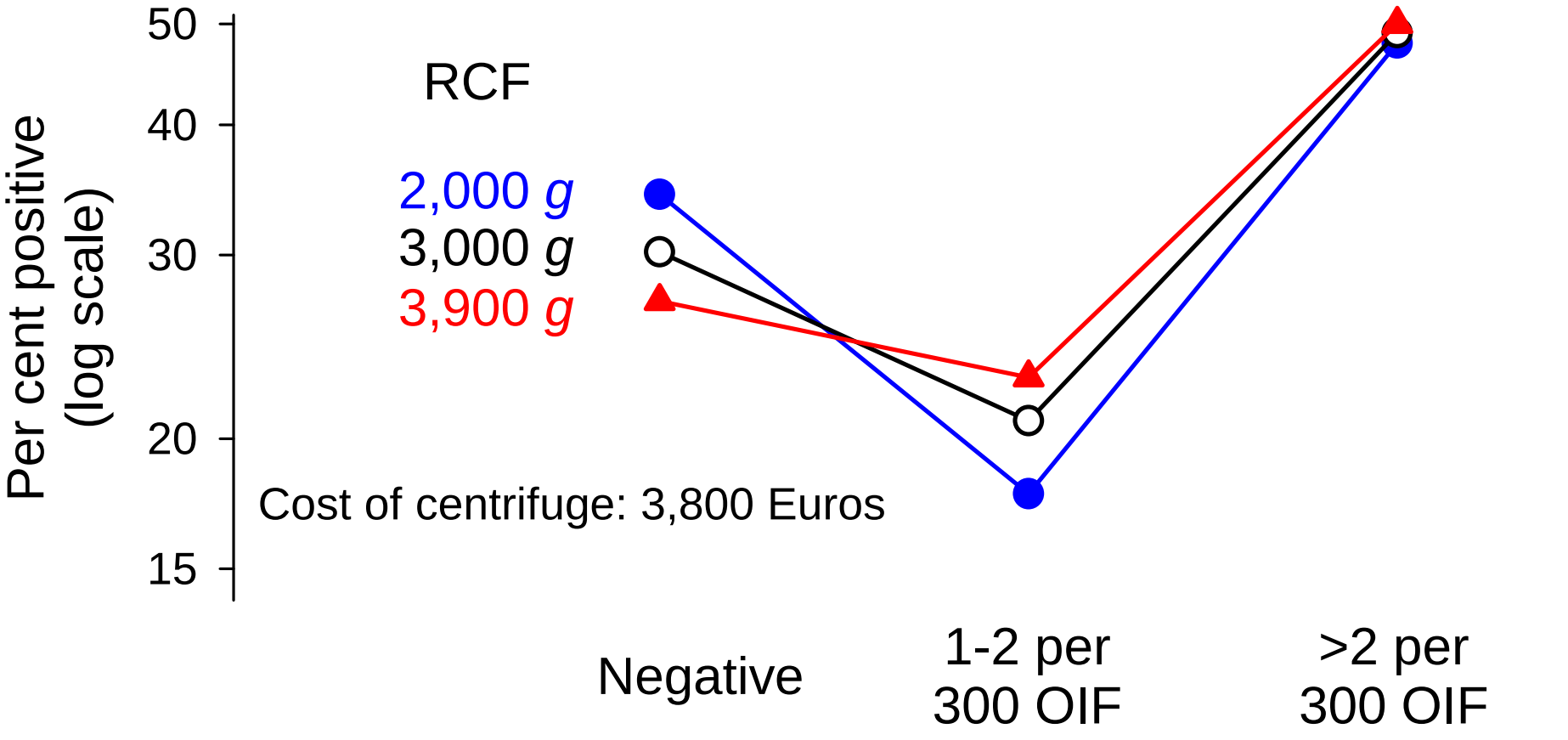
Toman K. Tuberculosis case-finding and chemotherapy. World Health Organization, Geneva, 1979

Distribution of Graded Results among Sputum Smear Microscopy Positive Cases, by Country



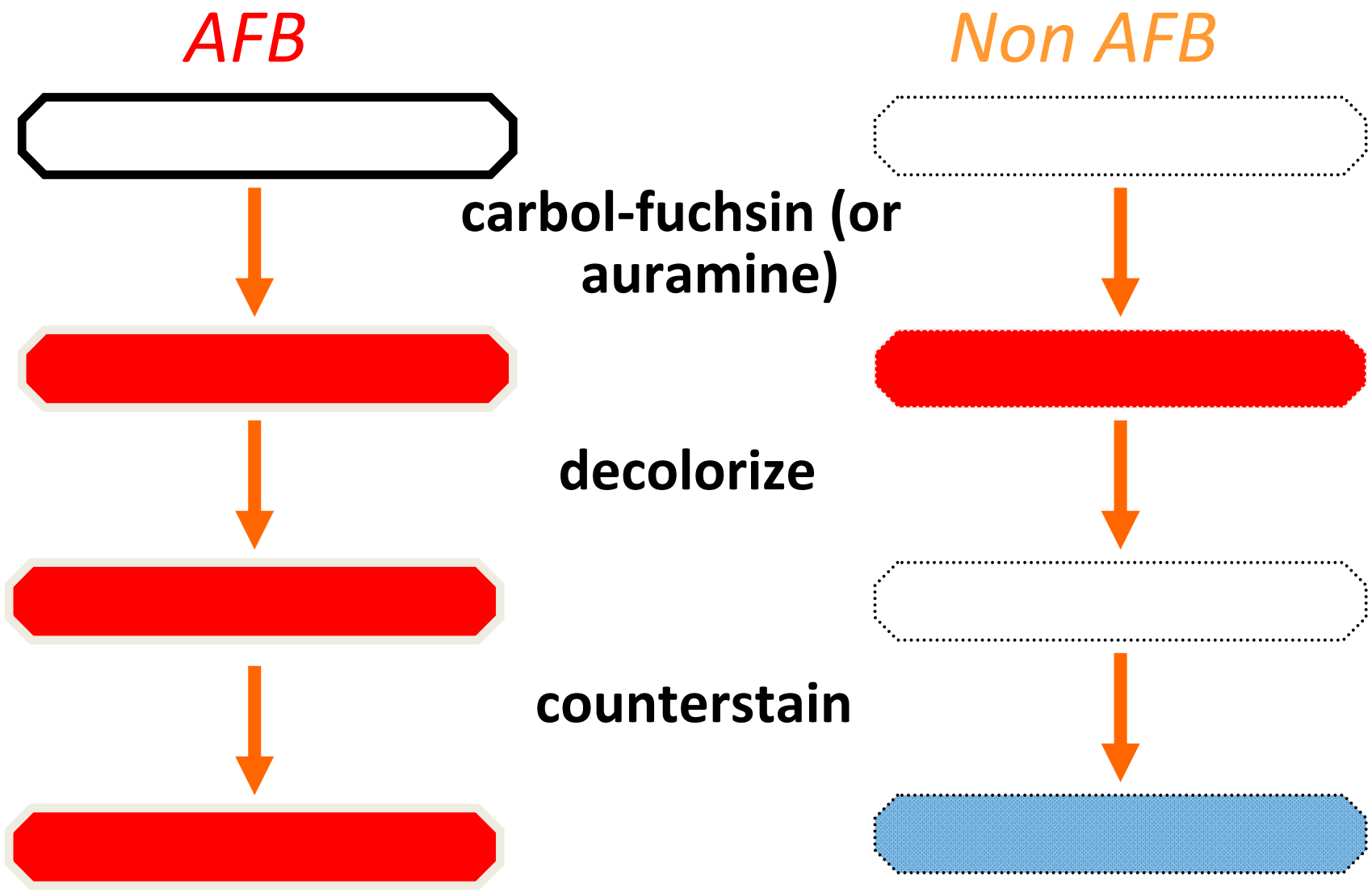
Unpublished data, Union Tuberculosis Laboratory Collaborative Study

Distribution of AFB Smear Results Among Culture-Positive Specimens, by Relative Centrifugal Force, 15 Minutes



Ratnam S, et al. J Clin Microbiol 1986;23:582-5

Principle of staining



Slide courtesy: Van Deun A, unpublished lecture notes, Union International Tuberculosis Course, Arusha, November 2009

Principles in two types of microscopy

Staining	Bright-field microscopy	Flurorescence microscopy
Primary stain (stains everything)	Fuchsin	Auramine
Decolorant (destains everything except mycobacteria)	Hydrochloric acid *or* Sulfuric acid	Hydrochloric acid
Counter-stain (stains everything except color-saturated mycobacteria)	Methylene blue	Potassium permanganate *or* Ink

The “Ziehl-Neelsen” staining technique: an experimental path to optimization, ready and all set since 1882

Contributor	Contribution
Robert Koch	Primary stain: methylene blue, alkaline potassium hydrate as mordant, vesuvium as both decolorant and counterstain
Paul Ehrlich	Fuchsin as primary stain, alkaline alinine as mordant, nitric acid as decolorant, and proposal of a blue counterstain
Franz Ziehl	Replace mordant with phenol
Friedrich Neelsen	Combine the best of all: primary and counterstain from Ehrlich, mordant from Ziehl, and replacing decolorant with sulphuric acid

Bishop P J, Neumann G. The history of the Ziehl-Neelsen stain. Tubercle 1970;51:196-206

Münch Med Wschr 1938;85:1066-8

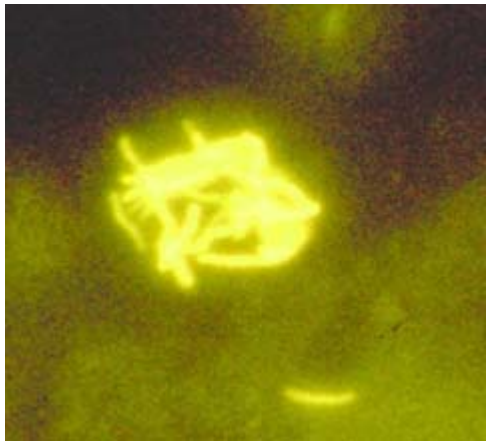
Technik.

Aus dem Hygienischen Institut der Universität Köln.
(Direktor: Prof. Reiner Müller.)

Fluoreszenzfärbung von Tuberkelbakterien mit Auramin.

Von Paul K. H. Hagemann.

Man läßt 15 Minuten eine 5proz. Phenolum liquefactum enthal tende Auraminlösung (1 : 1000 in dest. Wasser; nach dem Phenol zusatz gut durchschütteln) auf den in der Flamme fixierten Aus strich einwirken, spült kräftig mit Leitungswasser ab, differenzier mit HCl-Brennspiritus (1000 cm³ Brennspiritus; 4 cm³ konz. reine HCl des DAB; 4 g NaCl) 3 Min., wobei nach 1½ Min. der HCl Brennspiritus erneuert wird; daraufhin abermaliges kräftiges Ab spülen mit Leitungswasser. Eine Gegenfärbung unterbleibt. Die Prä parate sehen makroskopisch wie ungefärbt aus.



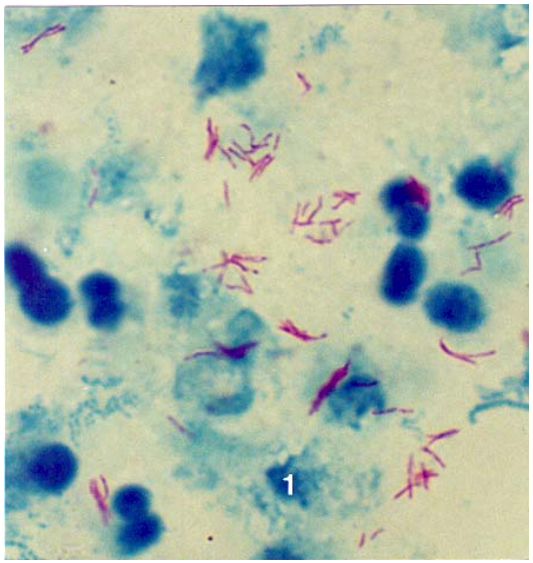
Stained with above recipe (HLR, Med Pol, ZH, 1978), but added potassium permanganate for background quenching

Magnification: 1,000x, oil immersion

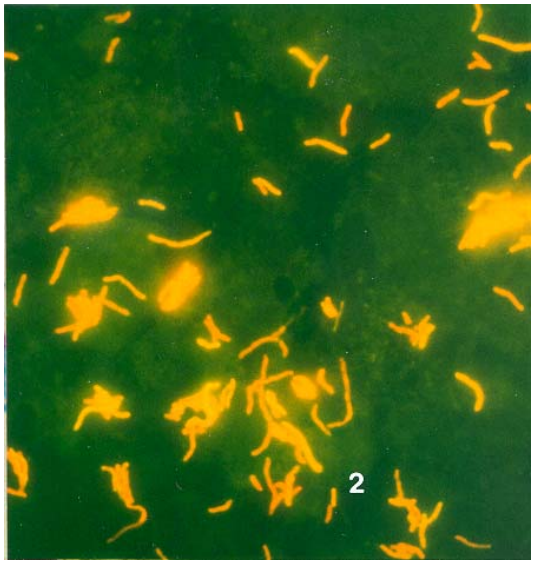
(Granulation disappears in photo due to long exposure time)

Appearance of AFB in bright-field and fluorescence microscopy

Ziehl-Neelsen



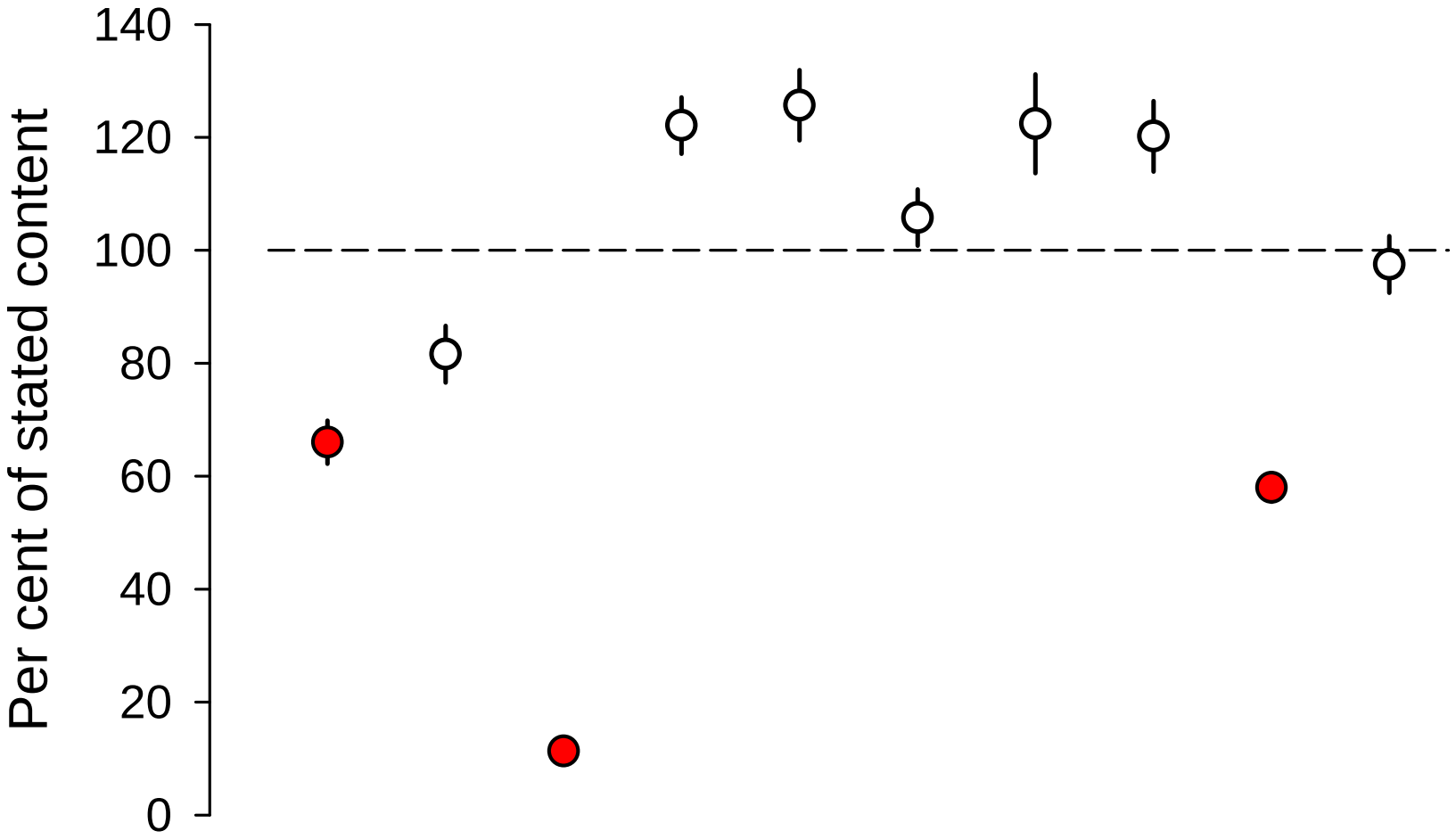
Fluorescence



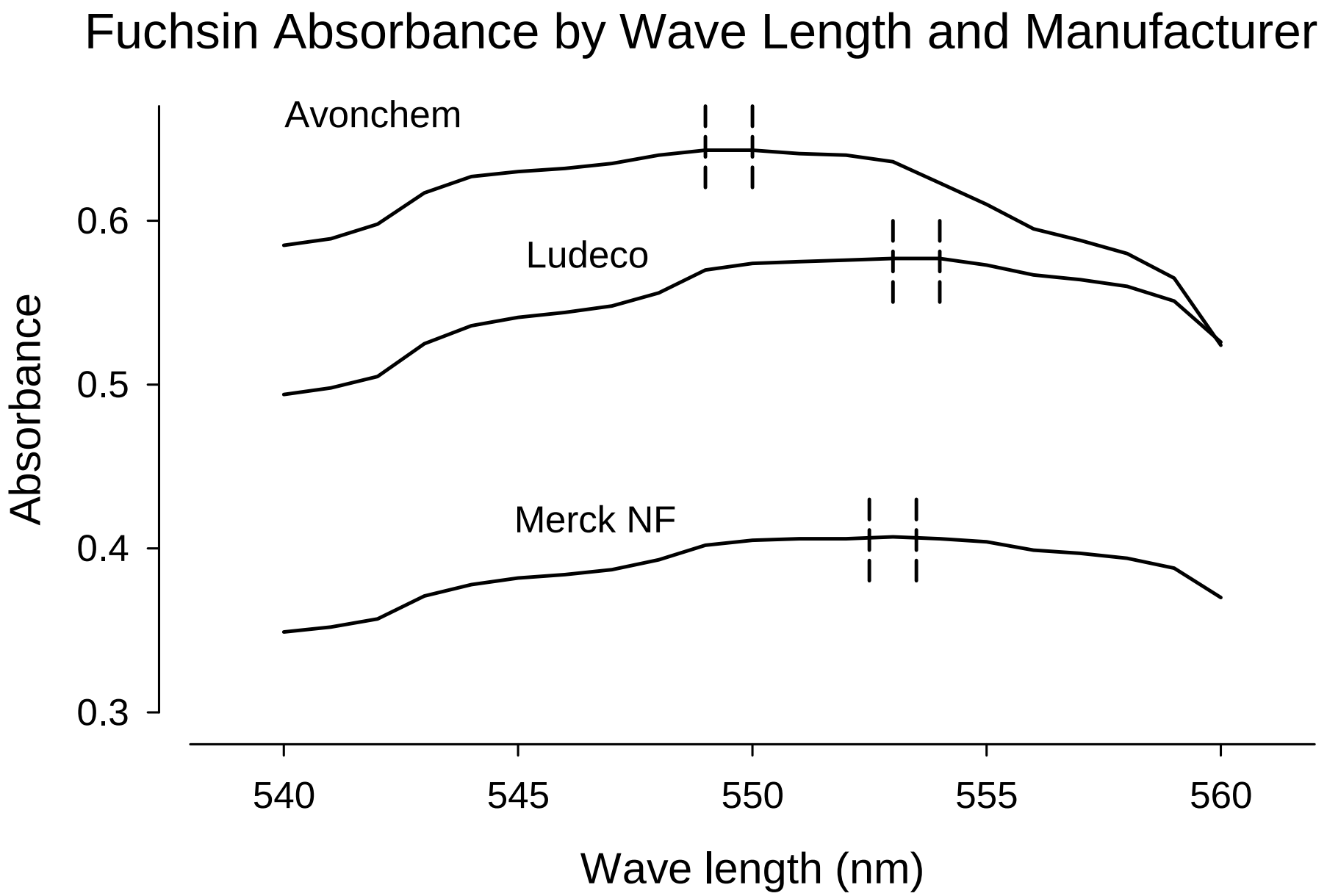
Picture courtesy:

Kim SJ. Unpublished lecture notes, Union Hanoi course, 4 September 2008

Variation in fuchsin content in 10 samples collected during routine field visits, China, 2005-2006



Zhao YL, et al. Int J Tuberc Lung Dis 2009;13:126-9

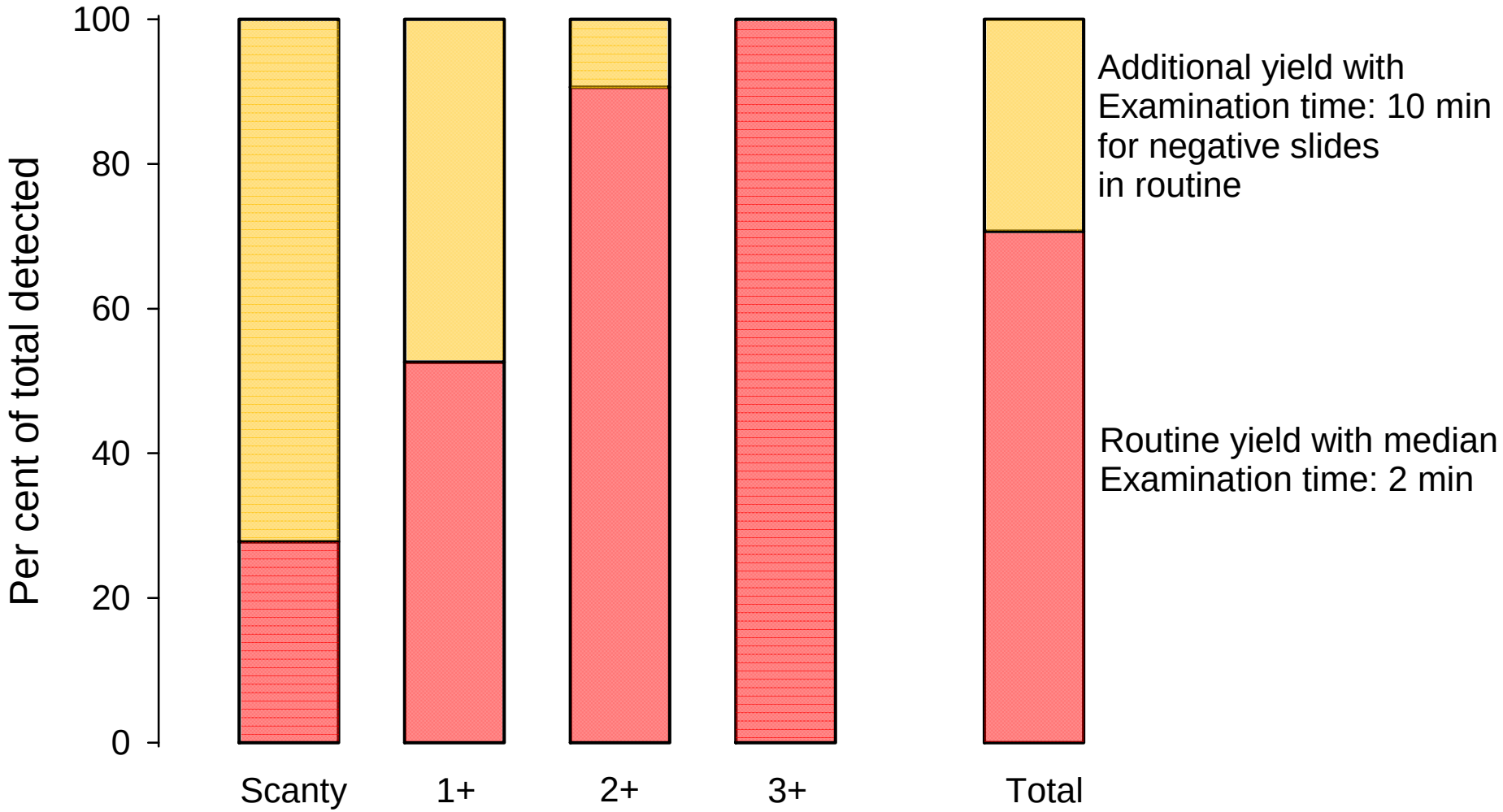


Data courtesy: Van Deun A, unpublished experiments, 2008

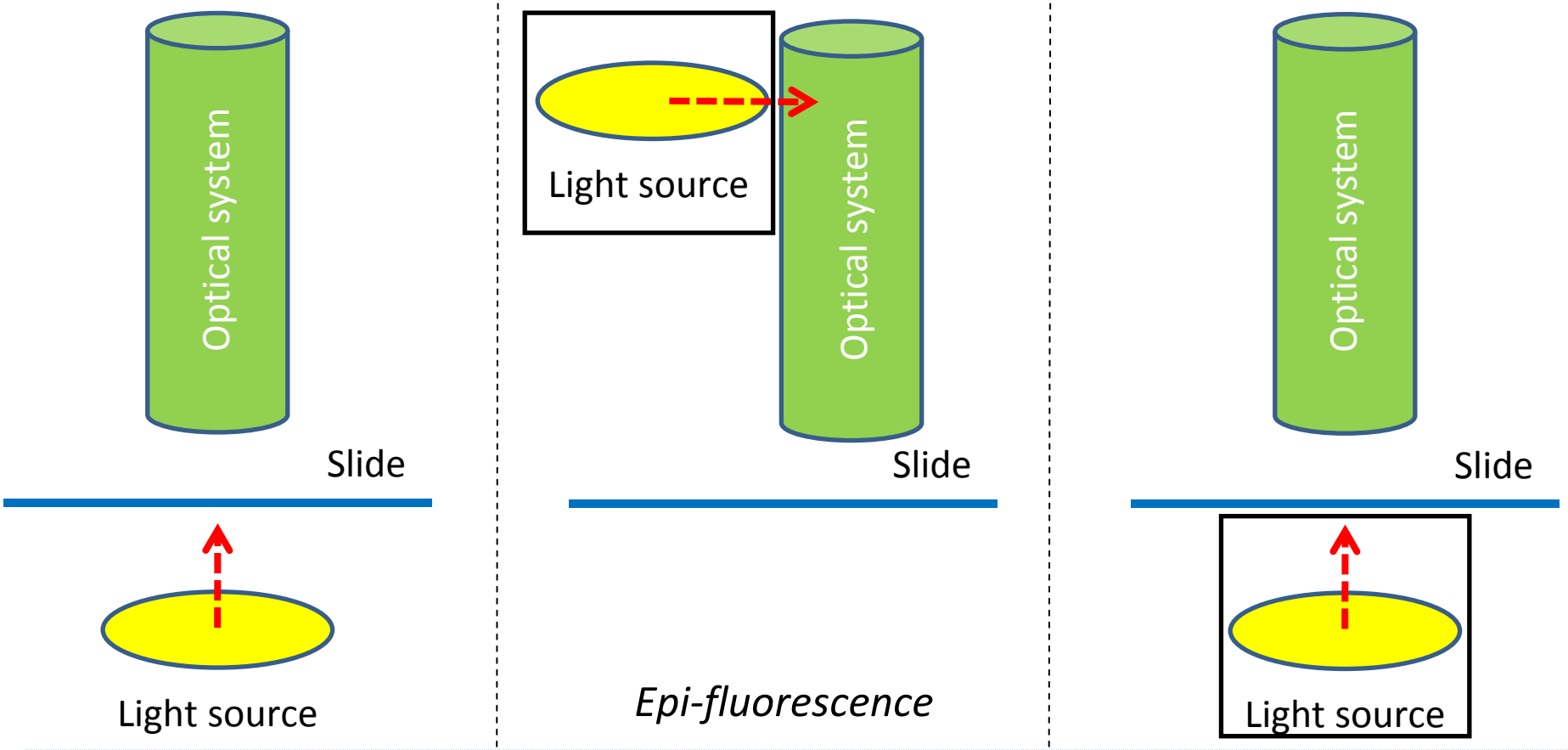
Visualizing the fuchsin content of different stains by dilution

Slide courtesy: Kam KM. Unpublished lecture notes, Union International Tuberculosis Course Hanoi, Viet Name, September 2008

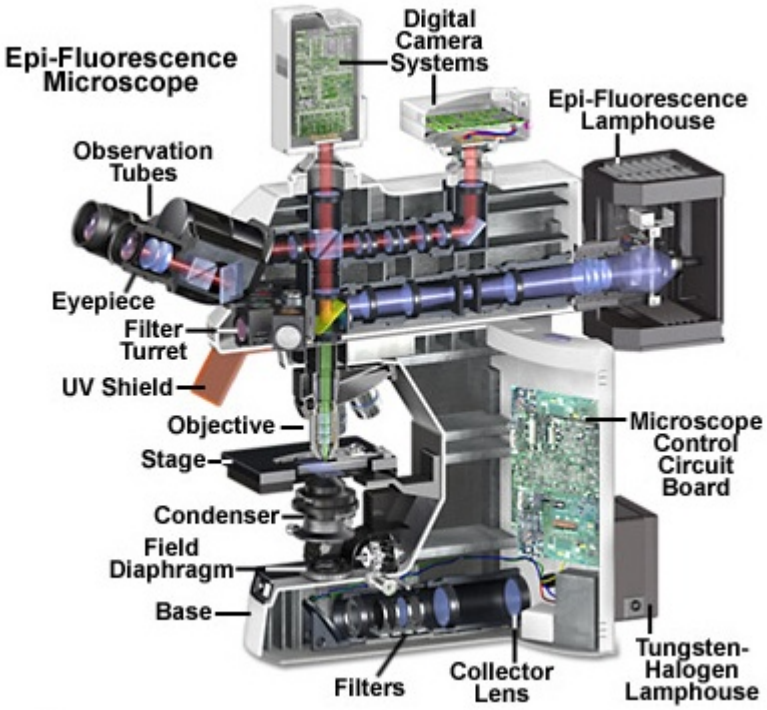
Investing sufficient time in sputum smear examination, Cameroon



Cambanis A, et al. Int J Tuberc Lung Dis 2007;11:40-5


Optical system
Slide
Light source
Light source
Optical system
Slide
Epi-fluorescence
Optical system
Slide
Light source
Bright field microscopy
Fluorescence microscopy
LED fluorescence microscopy
LED fluorescence microscopy

Principle of classical epi-fluorescence
(Example: Nikon system)



Principle of “add-on” LED module
in transmission mode
(Example: Fraen system)

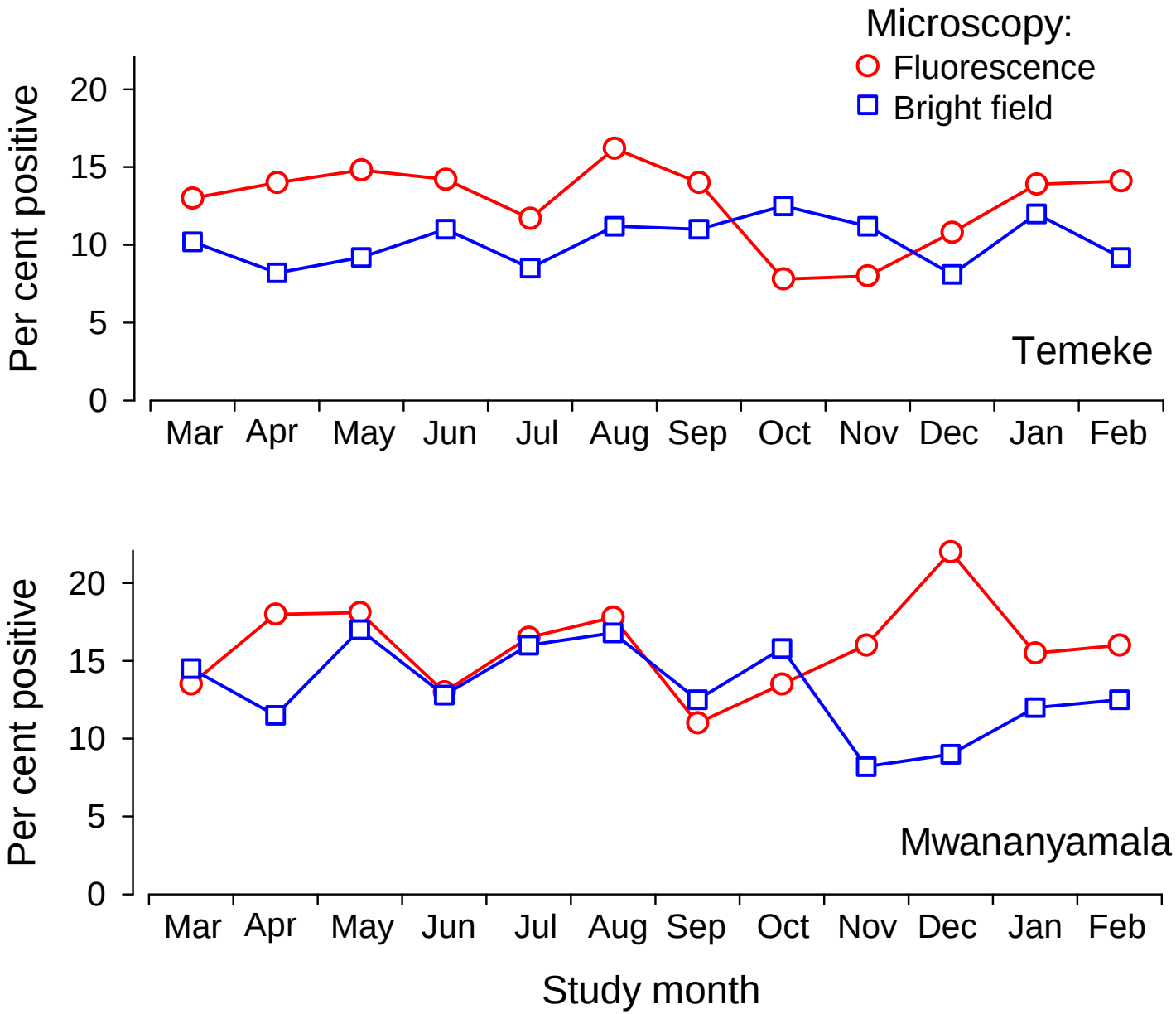
Added to an existing bright-field microscope without interference with the existing white-light function

Classical vapor lamp versus light emitting diode fluorescence microscopy

Readings using LED	Readings using mercury vapor lamp					
	Negative	Scanty	1+	2+	3+	Total
Negative	396	6	2	0	0	404
Scanty	1	7	4	0	0	12
1+	1	1	23	2	0	27
2+	0	0	2	15	4	21
3+	0	0	0	4	24	28
Total	398	14	31	21	28	492

Hung NV, et al. Lancet Infect Dis 2007;7:238-9

Yield from bright field versus fluorescence (LED) microscopy in two districts, Tanzania, 2007-2008

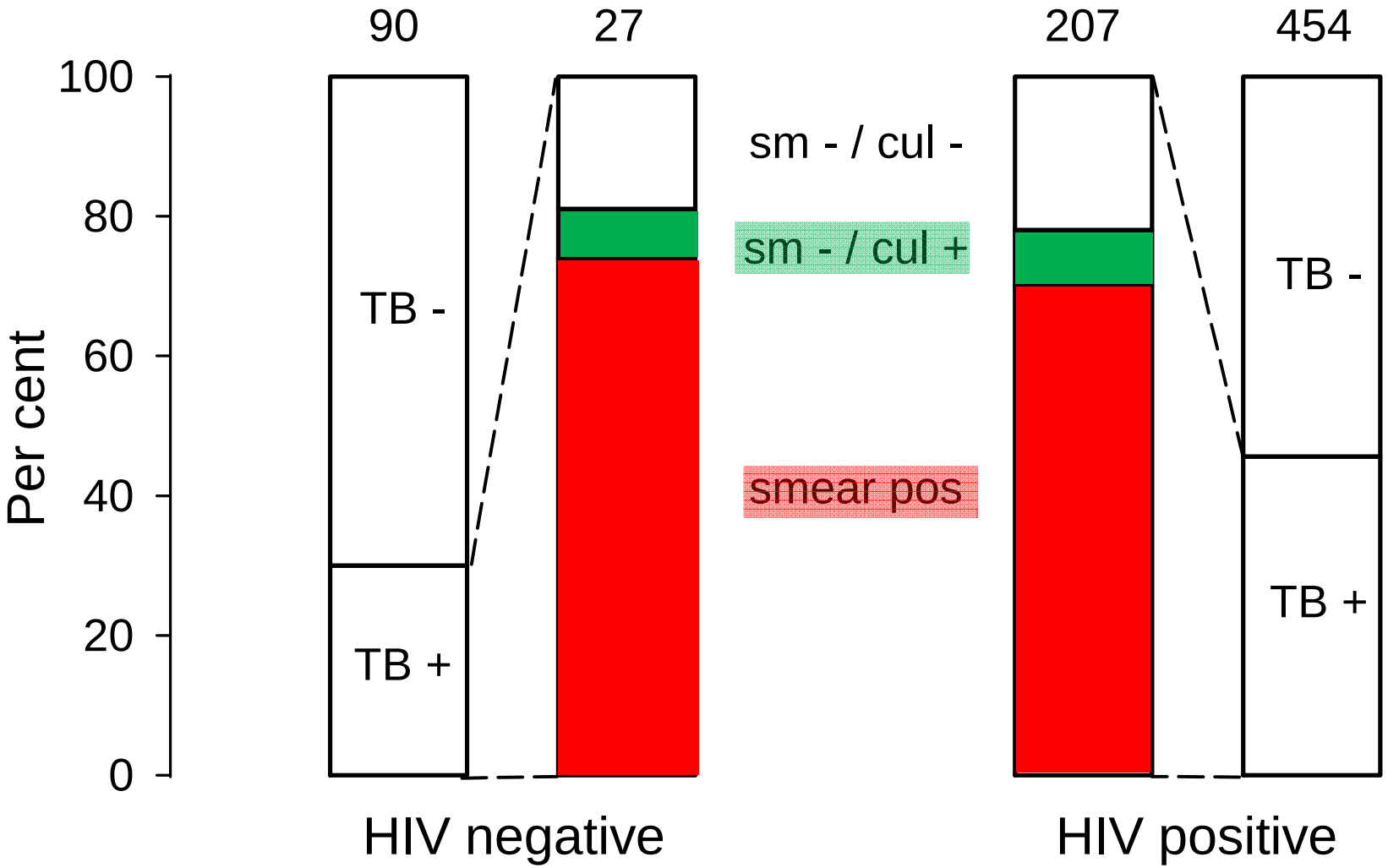


Van Deun A, et al. Int J Tuberc Lung Dis 2008;12:1009-14

Some differences between microscopy techniques

	Bright-field	Fluorescence (classic)	Fluorescence (LED)
Stain	Fuchsin	Auramine	Auramine
Time for proper examination	5 to 10 min	2 min	2 min
Microscope cost (€)	1,000	8,000	1,000
Module cost (€)	--	--	1,000
Lamp life (hr)	10,000	200	100,000
Maintenance cost	Low	Very high	Low
Acceptability	High	Often very low	High

Tuberculosis Among Primary Health Care Attendees with Prolonged Cough, Harare, Zimbabwe, 2003 (?)



Munyati SS, et al. Clin Infect Dis 2005;40:1818-27

Main issue with fluorescence microscopy: acceptability, not test operating characteristics!

o	Fluorescence microscopy not accepted in Zurich until 1980 (Policy change introduced by Pr Dr A von Graevenitz, technique by Dr M Salfinger)!
o	Fluorescence microscopy break-through acceptance in the US only after 1962 (Truant, et al), “selling” that combining auramine plus rhodamine was the solution to earlier non-acceptance – everybody forgot that quirky piece of history – there is “evidence” and “evidence”!
o	“Classical” fluorescence microscopy not accepted in Dar es Salaam in the 1990s
o	LED fluorescence microscopy in Dar es Salaam 2007-2008: ***microscopists refuse to return to Ziehl-Neelsen***