

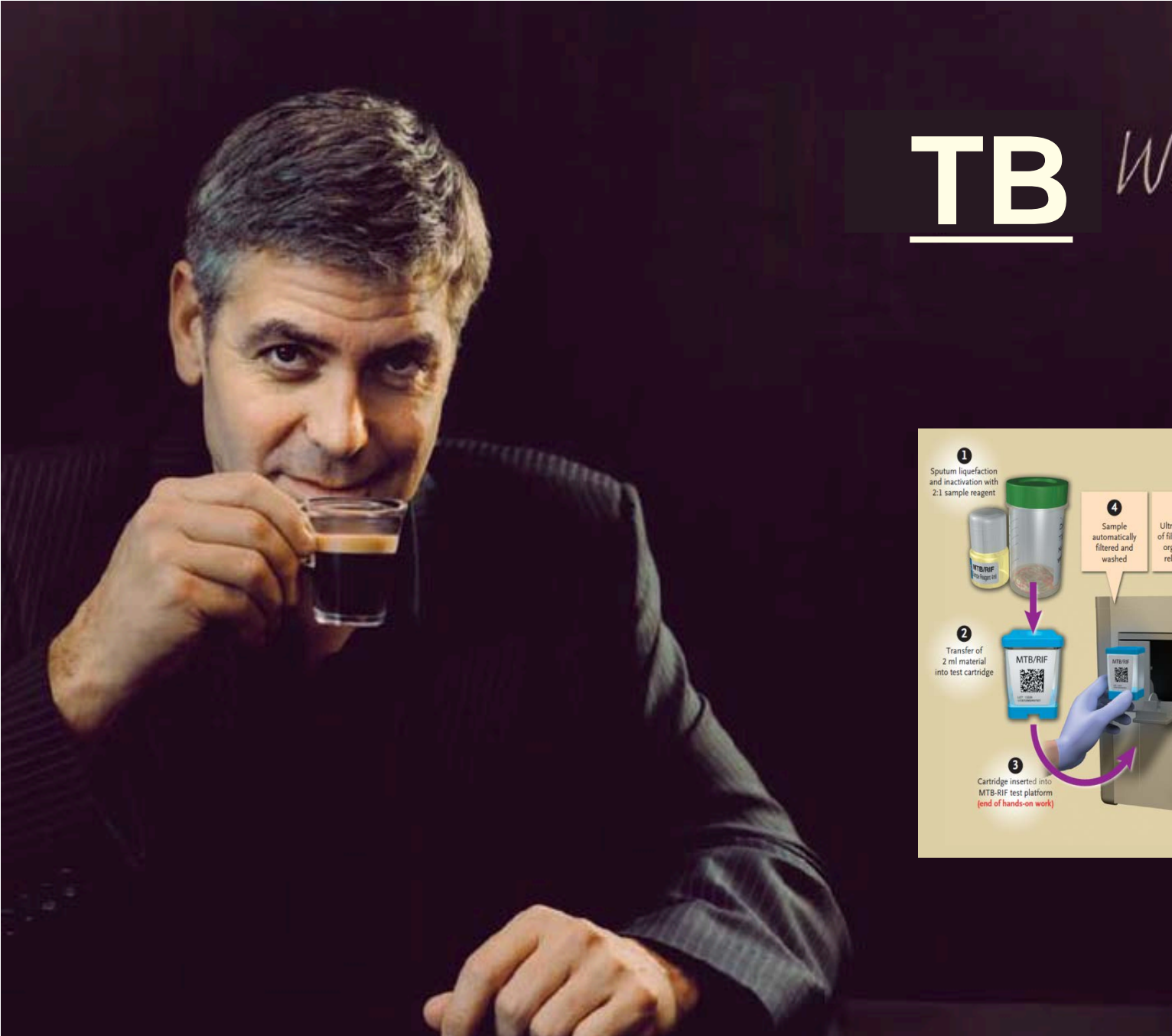
Kritische Fragen zum Beitrag der kombinierten PCR für den Nachweis von *M. tuberculosis* und der Identifizierung von Mutationen auf dem *rpoB*-Gen

Hans L Rieder

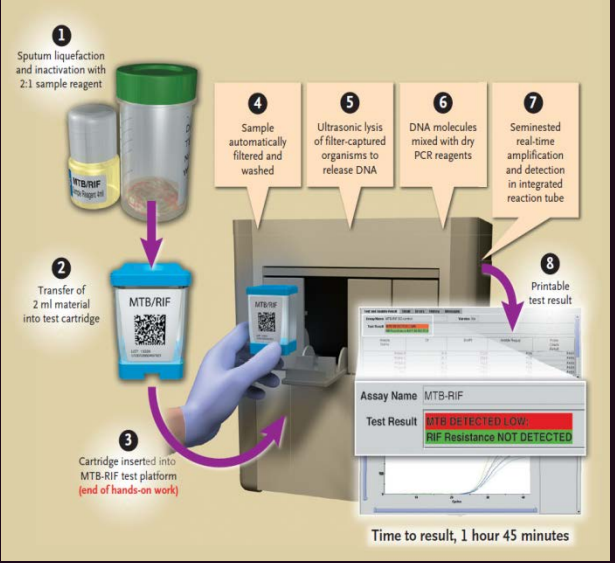
Union Internationale Contre la Tuberculose et les Maladies Respiratoires

Institut für Epidemiologie, Biostatistik und Prävention, Universität Zürich

Forschungszentrum Borstel – Leibniz-Zentrum für Medizin und Biowissenschaften



TB What else?



Slide courtesy: Christoph Lange, 29 Jan 2011

The Xpert MTB/RIF assay – basic tenets

The Xpert MTB/RIF assay gives up to two results:

- o The test as a diagnostic for tuberculosis identifies *M tuberculosis*-specific genome components (of dead or viable bacilli)
- o If the test is positive for *M tuberculosis*-specific genome components, it identifies mutations on the *rpoB* gene that are known to be associated with rifampicin resistance

Ergo:

If we want to use the assay as a diagnostic test system, we have to deal with the rifampicin susceptibility result that we also get if the test is positive for *M tuberculosis*-specific genome components

If we want to use the test as a test for rifampicin drug susceptibility, we have to consider whether the bacilli are alive or dead

Calculating the predictive value of a test result

Sensitivity: $a/(a+c)$

Prevalence: $(a+c)/N$

Specificity: $d/(b+d)$

Test result	Presence of characteristic		Total
	Yes	No	
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d=N

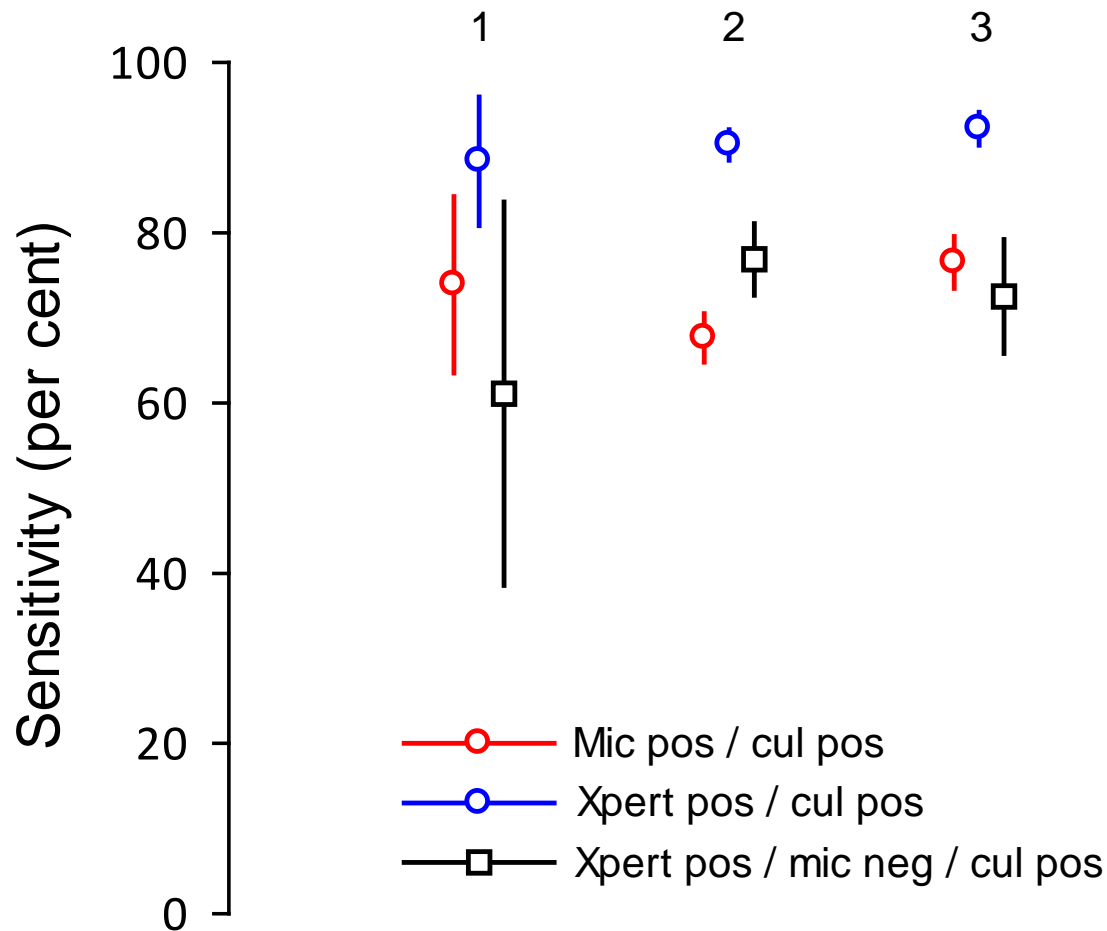
Predictive value of a positive test result: $a/(a+b)$

Considerations in the use of the Xpert MTB/RIF assay

Diagnosis of tuberculosis	Rifampicin resistance
Sensitivity	Sensitivity
Specificity	Specificity
Predictive value of a positive test	Predictive value of a positive test

Xpert MTB/RIF assay for the diagnosis of tuberculosis

Sensitivity of microscopy and Xpert MTB/RIF assay compared to culture in five studies



1) Rachow A, et al. *PLoS One* 2011;6(6): e20458:doi:10.1371/journal.pone.0020458

2) Boehme C C, et al. *Lancet* 2011;377:1495-505

3) Boehme C C, et al. *N Engl J Med* 2010;363:1005-15

4) Bowles E C, et al. *Int J Tuberc Lung Dis* 2011;15:988-9

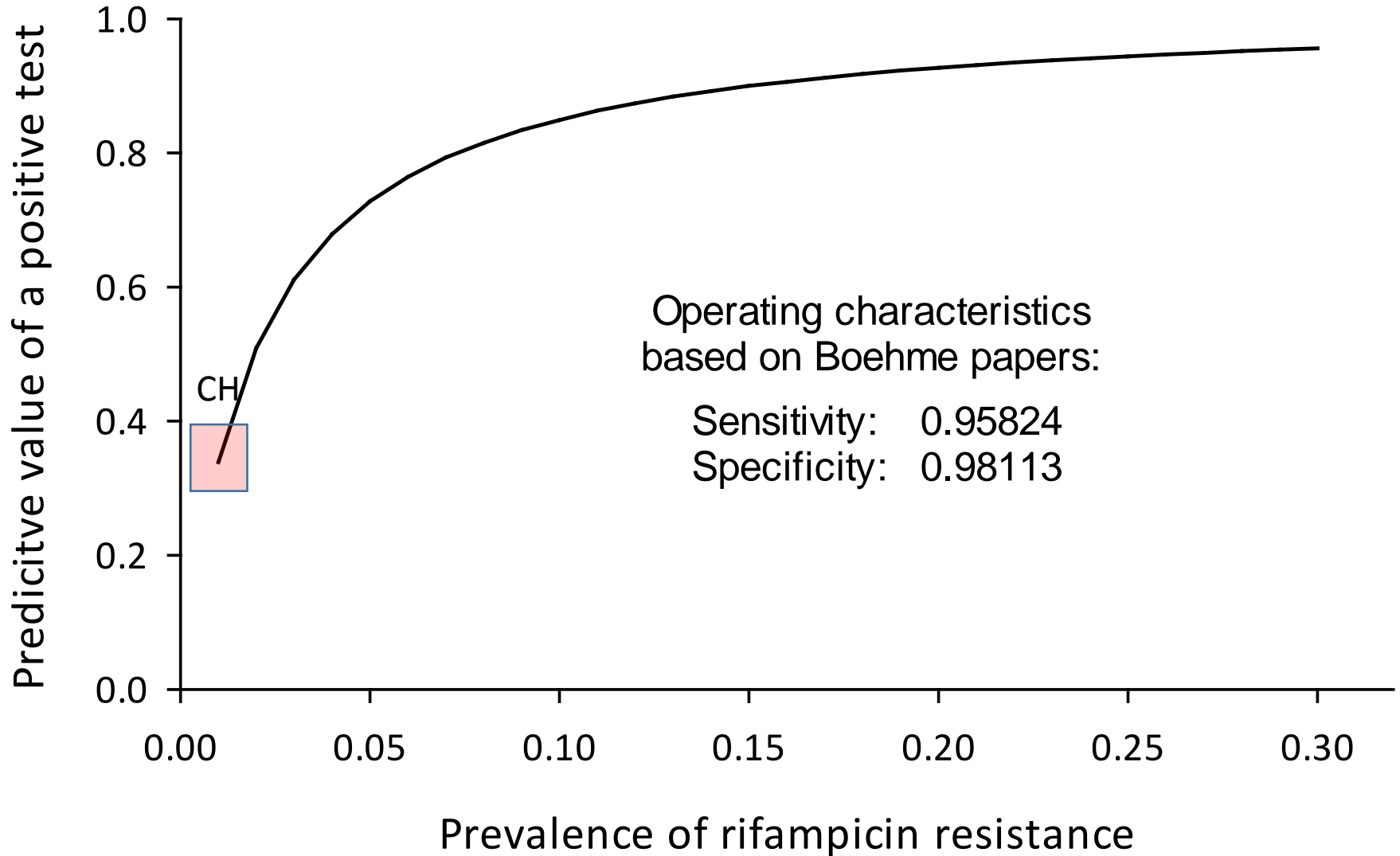
5) Helb D, et al. *J Clin Microbiol* 2010;48:229-37

Xpert MTB/RIF assay for the diagnosis
of rifampicin resistance

“When used to detect rifampicin resistance, Xpert MTB/RIF achieved a pooled sensitivity of 95% (95% CrI, 90–97%) (17 studies, 555/2624 total specimens) and a pooled specificity of 98% (95% CrI, 97–99%) (24 studies, 2414 specimens, including true negatives and false positives)”

*World Health Organization.
World Health Organization Document 2013;WHO/HTM/TB/2013.14:1-89*

Predictive value of a "rifampicin resistant" result with Xpert MTB / RIF, given prevalence of rifampicin resistance



Boehme CC, et al. N Engl J Med 2010;363:1005-15

Boehme CC, et al. Lancet 2011;377:1495-1505

Test system 1 (e.g. liquid phenotypic testing)

	Resistance				
Test	yes	no	Total		
pos	5	0	5	Sens	0.50
neg	5	90	95	Spec	1.00
Total	10	90	100		

	Truth		
	Resistance		
Test	yes	no	Total
pos			
neg			
Total	10	90	100

Test system 2 (e.g. genotypic testing)

	Resistance				
Test	yes	no	Total		
pos	8	0	8	Sens	0.80
neg	2	90	92	Spec	1.00
Total	10	90	100		

Test system 1 is made gold standard

	Resistance				
Test	yes	no	Total		
pos	4	5	8	Sens	0.80
neg	1	90	92	Spec	0.95
Total	5	95	100		

Rifampin Resistance Missed in Automated Liquid Culture System for *Mycobacterium tuberculosis* Isolates with Specific *rpoB* Mutations

Leen Rigouts,^{a,b} Mourad Gumusboga,^a Willem Bram de Rijk,^a Elie Nduwamahoro,^a Cécile Uwizeye,^a Bouke de Jong,^a Armand Van Deun^a

Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium^a; University of Antwerp, Antwerp, Belgium^b

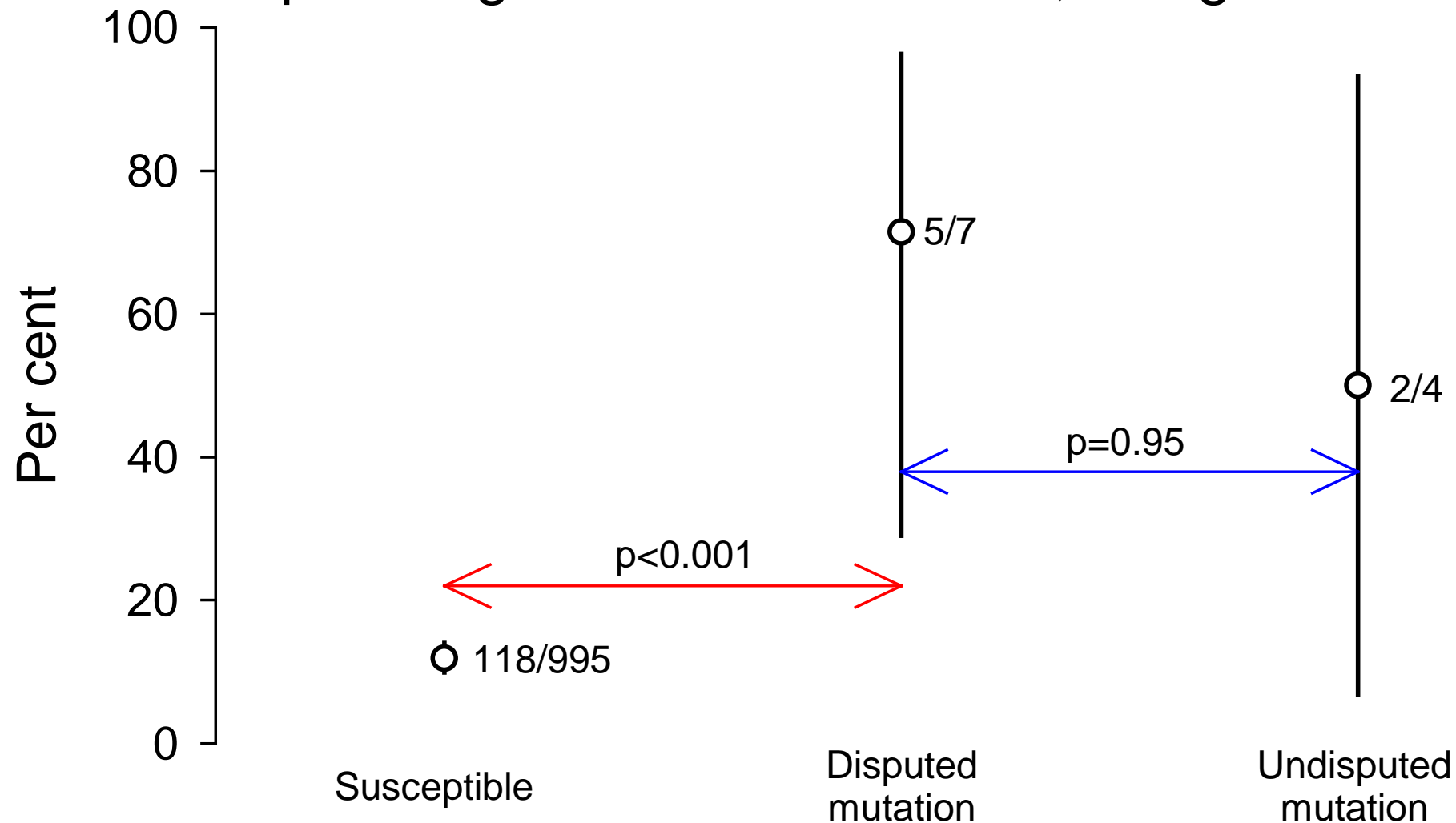
Rifampin Drug Resistance Tests for Tuberculosis: Challenging the Gold Standard

Armand Van Deun,^{a,b} Kya J. M. Aung,^c Valentin Bola,^d Rossin Lebeke,^d Mohamed Anwar Hossain,^c Willem Bram de Rijk,^a Leen Rigouts,^a Aysel Gumusboga,^a Gabriela Torrea,^a Bouke C. de Jong^a

Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium^a; International Union Against Tuberculosis and Lung Disease, Paris, France^b; Damien Foundation Bangladesh, Banani, Dhaka, Bangladesh^c; Coordination Provinciale Lèpre/Tuberculose, Kinshasa, Democratic Republic of Congo^d

Liquid culture phenotypic drug susceptibility testing systems miss some mutations that confer rifampicin resistance

Unfavorable treatment outcome and *rpoB* sequencing result for mutations, Bangladesh



Van Deun A, et al. Int J Tuberc Lung Dis 2015;19:185-90

A general principle:

Do not try to confirm a result of a first test with a second test that has a lower sensitivity than the first test.

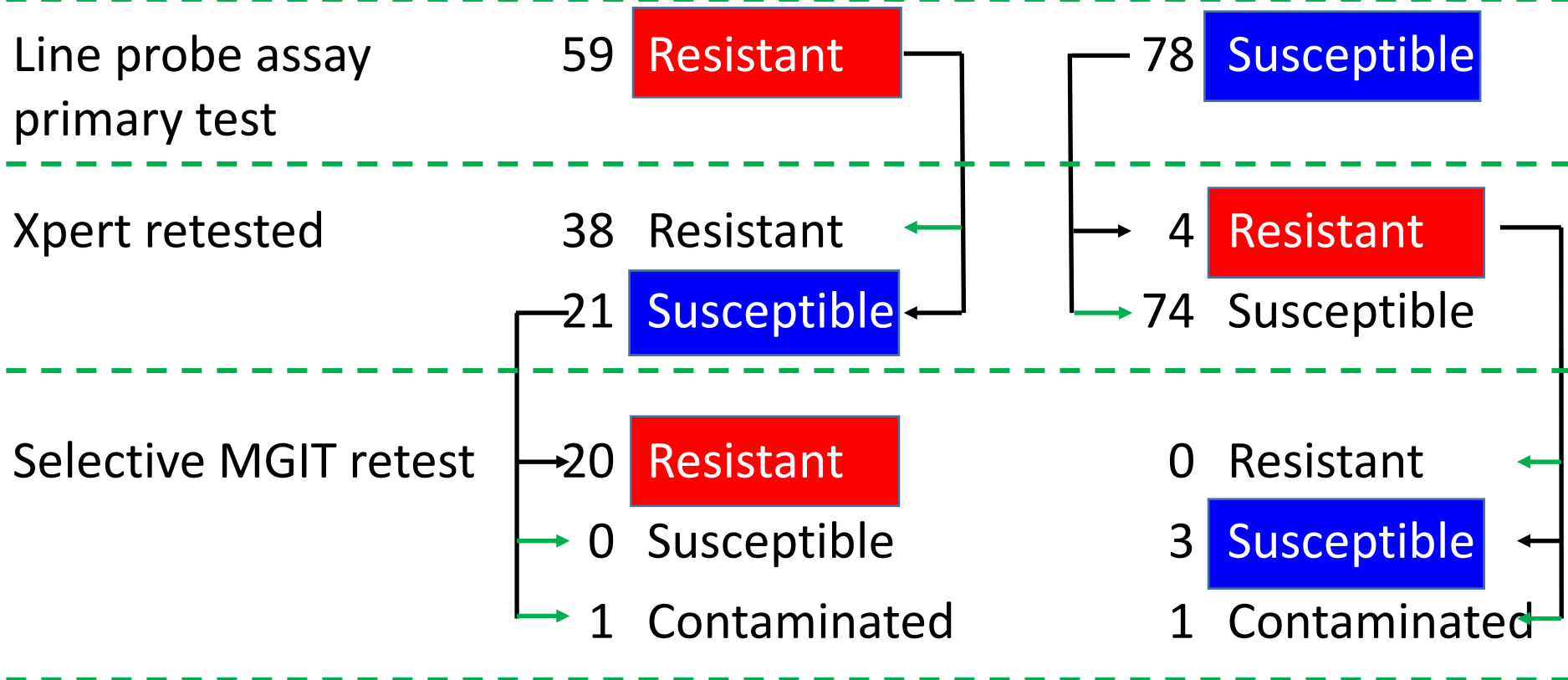
Example: do not try to confirm a scanty positive fluorescence microscopy result by bright-field microscopy

Specifically for the Xpert MTB/RIF assay:

Evidence is accumulating that Xpert MTB/RIF has a higher sensitivity for detecting rifampicin resistance than the automated liquid MGIT system (the same might not be true for drug susceptibility testing on solid media).

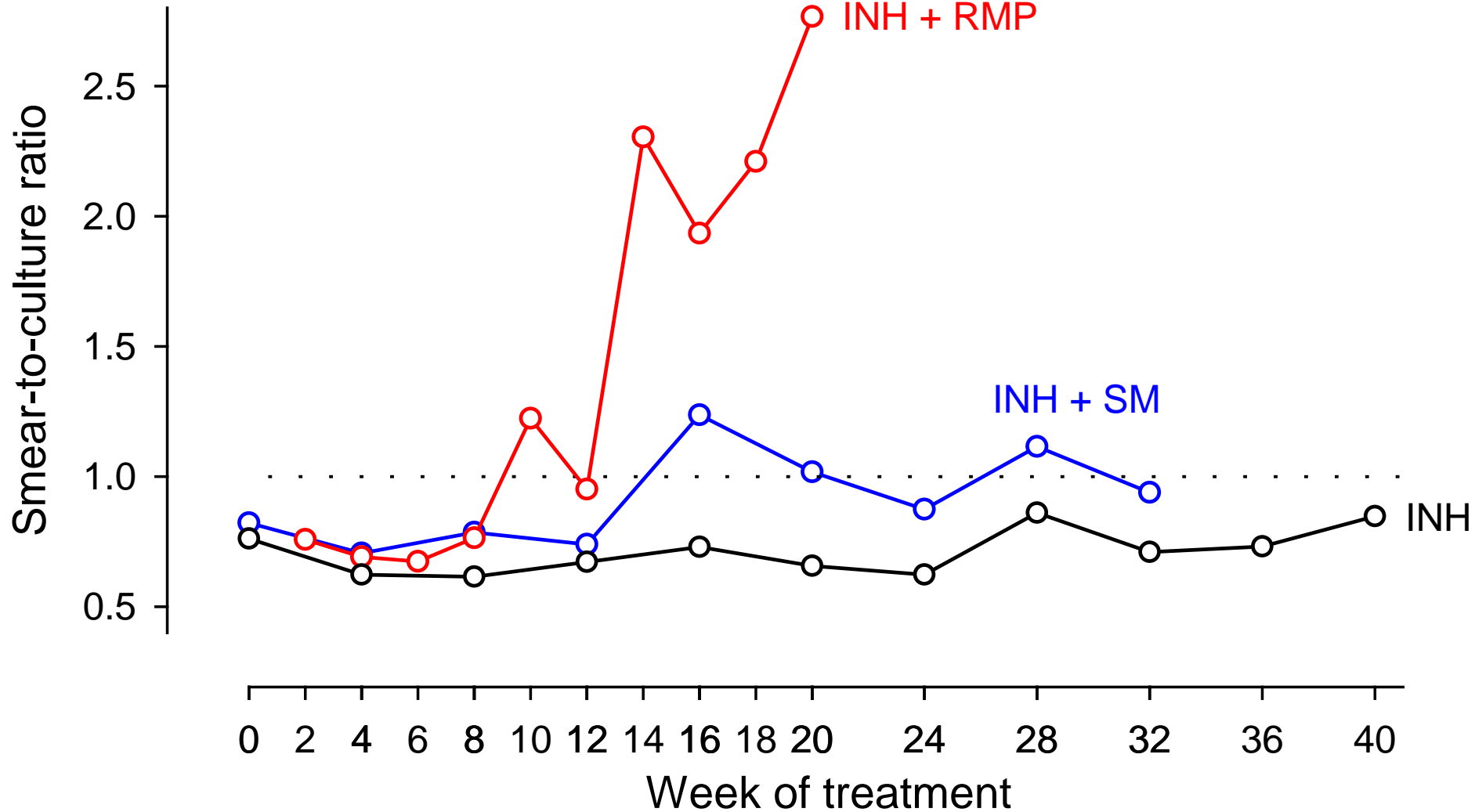
Therefore, to confirm an Xpert MTB/RIF rifampicin-resistant test result, it is probably best to repeat the Xpert MTB/RIF assay on another specimen rather than using a liquid phenotypic test system.

Bad news from the All India Institute of Medical Sciences about Xpert rifampicin test sensitivity?



Should Xpert MTB/RIF ever be used for follow-up examination during chemotherapy?

Smear-to-Culture Ratio During Chemotherapy by Treatment Regimen, USPHS Trials



Mount FW, et al. Am Rev Tuberc 1953;68:264-9
Mount FW, et al. Am Rev Tuberc 1954;70:521-6
Newman R, et al. Am Rev Respir Dis 1974;109:216-32

Is there any indication of Xpert MTB/RIF as an examination tool during chemotherapy?

- o No indication in a patient who is well responding and has negative sputum smear microscopy examinations after about 3 months of chemotherapy
- o If a sputum smear microscopy examination is positive after about 3 months of chemotherapy, an Xpert MTB/RIF test can be useful:
 - o 1) If *M tuberculosis* is identified, this is frequently meaningless (DNA from non-viable bacilli is amplified by PCR)
 - o 2) Solely the rifampicin susceptibility test result is relevant:
 - if susceptible, then the assay result can be disregarded;
 - if the test result shows rifampicin resistance, the patient must be switched to a regimen containing second-line drugs.

Danke für Ihr Interesse!