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FOR MYCOBACTERIAL
INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

PCR and direct amplification for tuberculosis diagnosis

Emmanuelle CAMBAU

University Paris Diderot,

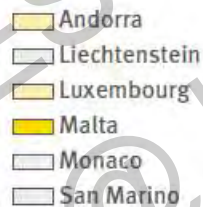
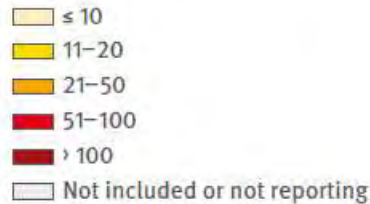
APHP, Saint Louis-Lariboisière Hospital,

Paris, France

**Educational Workshop 05- ECCMID 2015 Copenhagen
The impact of diagnostics on clinical tuberculosis management**

Tuberculosis in Europe - 2013

Map 3: TB notification rates, new TB cases and relapses per 100 000 population, European Region, 2013



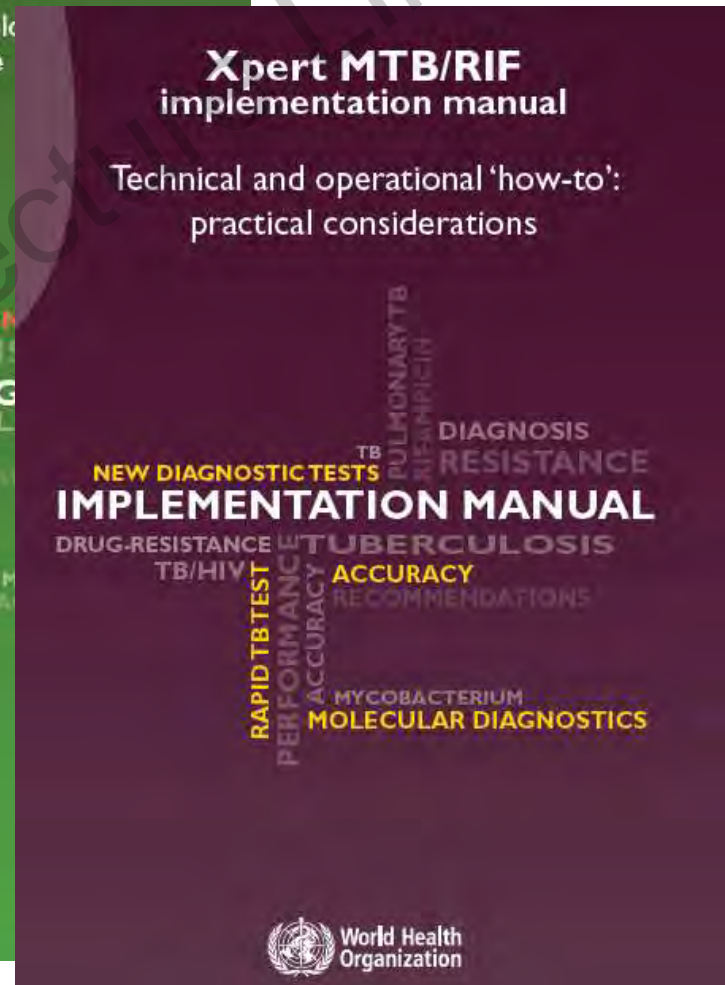
http://ecdc.europa.eu/en/publications/surveillance_reports/2015

Nucleic acid amplification direct testing (NAAT) for detection of *M. tuberculosis* complex

- from 1990 onwards using
 - gene (Hermans JCM 1990)
 - RNA (Boddinghaus JCM 1990)
 - IS6110 (Thierry JCM 1990)
- Pubmed
 - 2850 papers on diagnosis: 150 median per year
 - 150 review papers, 8 meta-analyses
- Several recommendations
 - ATS; CDC; WHO
- Several commercial kits and home made protocols

ATS 1997, Ieven CMR1997, Sarmiento JCM2003, Dinnes HTA 2007,
Greco JCM 2009; MMWR 2009;58; Lawn LID2013

WHO publications 2014



Xpert® MTB/RIF assay for direct diagnosis of pulmonary tuberculosis

Steingart et al. Cochrane Database Syst Rev. 2013 Jan 31;1

Steingart et al. Cochrane Database Syst Rev. 2014 Jan 21;1

- 22 studies, 8998 participants
- **Sensitivity: 89%** (95%CrI 85% - 92%)
- **Specificity: 99%** (95% CrI 98% - 99%)

Interpreting results of studies on NAAT/PCR for tuberculosis

- What is sensitivity? And how much is it important
- What is specificity? And how much is it important
- How are predictive values calculated?
 - Positive predictive value
 - Negative predictive value
- Concordance with smear and culture results

Sensitivity of NAAT

Direct Detection of *Mycobacterium tuberculosis* in Sputum by Polymerase Chain Reaction and DNA Hybridization

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Emory University Hospital,¹ The Emory Clinic,² and Department of Pathology and Laboratory Medicine,
Emory University School of Medicine,³ Atlanta, Georgia 30322; Grady Memorial Hospital, Atlanta,
Georgia 30355⁴; and Hansen's Disease Laboratory, Division of Bacterial Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia 30333⁵

TABLE 1. Correlation of acid-fast bacillus smear quantitation and PCR results for 124 sputum specimens containing *M. tuberculosis*

Smear quantitation (no. of specimens)	No. (%) of specimens PCR positive	
4+ (75).....	74 (99)	Smear pos
3+ (12).....	11 (92)	
2+ (17).....	16 (94)	
1+ (6).....	4 (67)	
Negative (14).....	8 (57)	Smear neg

NAAT sensitivity for TB diagnosis

= We would like that all TB patients with Smear-positive specimens are NAAT+

- Cochrane review
 - Pooled sensitivity = **98%** (95% CrI 97- 99%)
- WHO study group review
 - Pooled sensitivity = **98%** (95%CrI 97-99%)

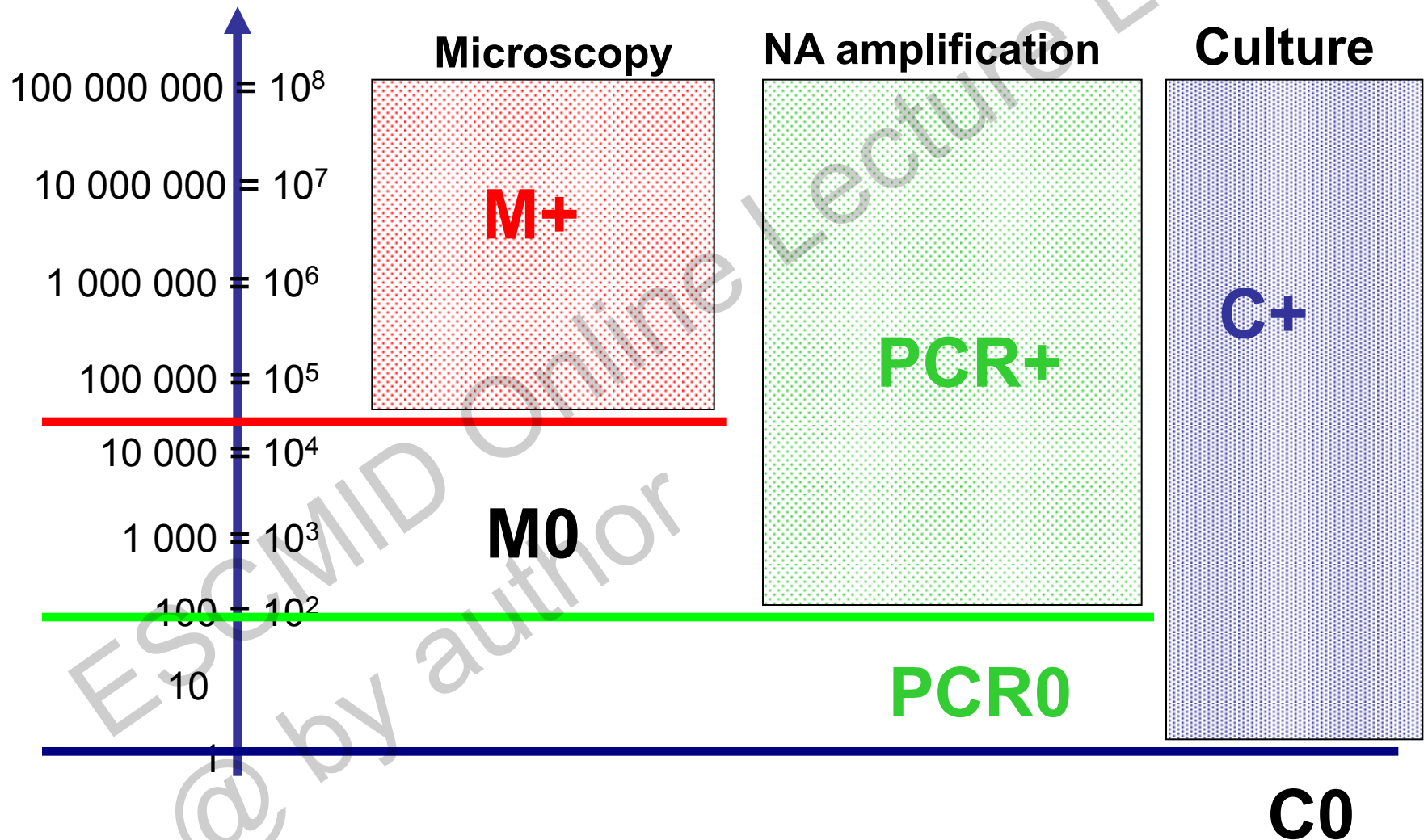
NAAT sensitivity for TB diagnosis

= We would like that even TB patients with Smear-negative specimens are NAAT+

- Cochrane review
 - Sensitivity : **67%** (95% CrI 60% -74%)
- LID Review (Lawn 2013)
 - Sensitivity : **75%** (range: 47% - 83%)
 - Extrapulmonary TB : **77%** (range 25%-97%)
- WHO expert group review
 - Sensitivity : **68%** (95% CrI 61%-74%)

Sensitivity of diagnosis tools for tuberculosis

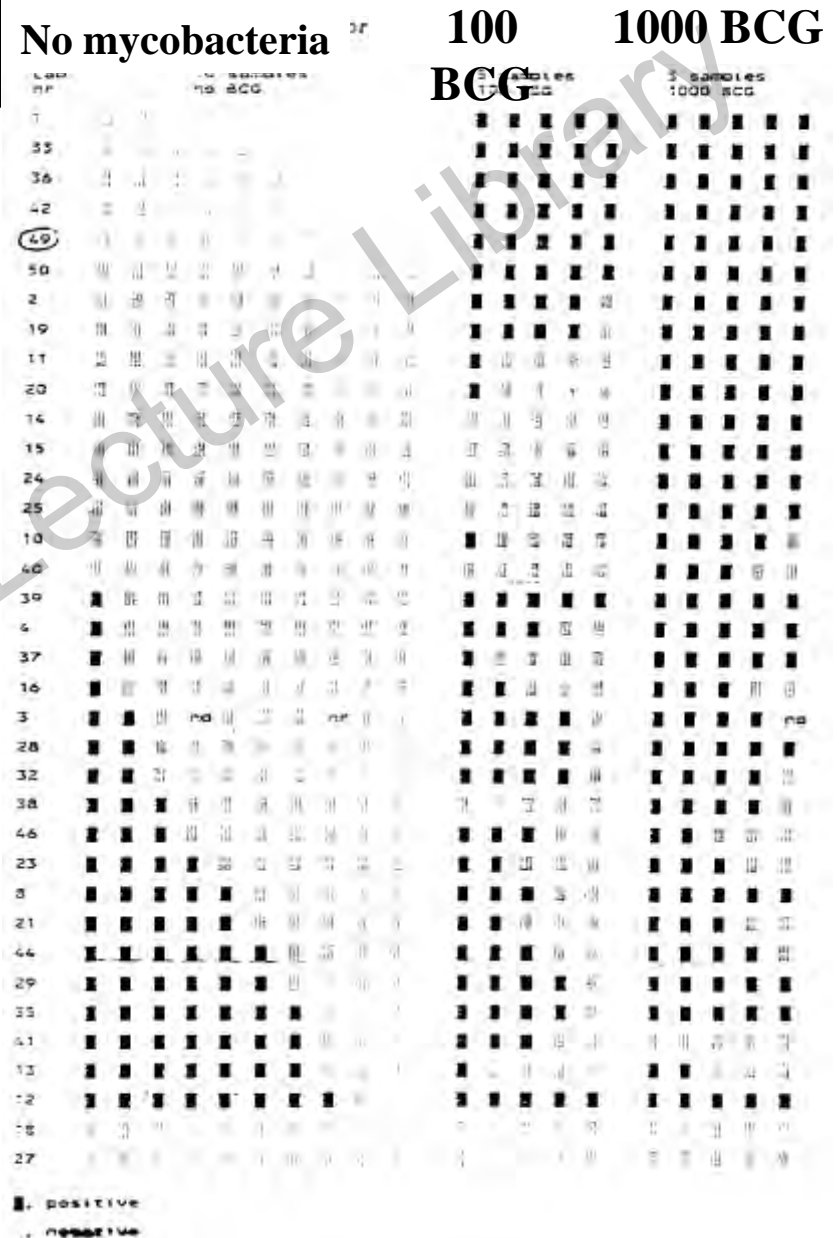
N per ml specimen



Specificity of NAAT

**Results of the
interlaboratory
study in detection
of *M.tuberculosis*
Noordhoek et al.
JCM 1996;34:2522-5**

- 30 expert laboratories
- 20 external quality controls
- 10 = no mycobacteria
- 5 = 100 BCG
- 5 = 1000 BCG



NAAT specificity for TB

= We would like that patients without TB are NAAT negative

- Cochrane review
 - Specificity : **98%** (95% CrI 97% to 99%)
- LID Review
 - Specificity : **98.6%** (range 88.9% - 100%)
- WHO study group review
 - Specificity : **99%** (95% CrI 98% to 99%)

Interpreting study results on NAAT/PCR in tuberculosis

	Patients tested		
	TB*	noTB	
Positive PCR test	A True positive	B False-positive	
Negative PCR test	C False-negative	D True-negative	
	Sensitivity $A / A + C$	Specificity $D / D + B$	

* Based on smear, culture or clinical signs?

Predictive values

- Are related to the prevalence of TB among the population tested by PCR
 - Interesting if the prevalence is not biased
- Positive predictive value (PPV) depends on specificity rate
 - High PPV if the TB prevalence is high ($> 10\%$)
- Negative predictive value (NPV) depends on sensitivity
 - High NPV if the prevalence is very low ($< 1\%$)

Interpreting study results on NAAT/PCR in tuberculosis

	Patients tested		
	TB	noTB	
Positive PCR test	A* True positive	B False-positive	PPV $A / A+B$
Negative PCR test	C False-negative	D True-negative	NPV $D / D+C$
	Sensitivity $A / A+C$	Specificity $D / D+ B$	

* Based on smear, culture or clinical signs?

Xpert® MTB/RIF assay for direct diagnosis of pulmonary tuberculosis and rifampicin resistance

Karen R Steingart¹, et al. Cochrane Database Syst Rev. 2014 Jan 21;1

- **22 studies for diagnosis, 8998 participants,**
- **2500 cases of TB : prevalence 33%**
- pooled sensitivity 89% (95% CrI 85% to 92%)
- pooled specificity 99% (95% CrI 98% to 99%)
- Negative smear microscopy
 - sensitivity 67% (95% CrI 60% to 74%)
 - specificity 99% (95% CrI 98% to 99%)
- Positive smear microscopy (65%)
 - sensitivity 98% (95% CrI 97% to 99%)

The prevalence (PV) varies depending on the specimen tested

TB forms	Prevalence
Smear-positive pulmonary (and non pulmonary) NTM infection can be also smear-positive	98% to 100%
Pulmonary smear-negative <ul style="list-style-type: none">• in occidental countries• In endemic countries• screened with XRay or other test	2 – 5% 10% 30%
Extra-pulmonary specimen (e.g. CSF, even if screened on the basis of leucocytes > 10/mm ³)	0.5%

Predictive values depends on the prevalence of TB

TB forms	PV	PPV	NPV
Pulmonary smear-positive NTM infection also smear-positive	98% to 100%	98% to 99.5%	90%
Pulmonary smear-negative <ul style="list-style-type: none"> • in occidental countries • endemic countries • screened with XRay or other test 	2% – 5% 10% 30%	34 % to 57%	97% to 99%
Extra-pulmonary specimen as CSF even if screened on the basis of leucocytes > 10/mm ³	0.5%	8%	99.7%

PV, prevalence; PPV, positive predictive value; NPV, negative predictive value

Number of cases diagnosed, missed, give false diagnosis, per prevalence rate

Test result	Number of results per 1000 patients tested (95% CrI) ¹		
	Prevalence 2.5%	Prevalence 5%	Prevalence 10%
True Positives	22 (21, 23)	45 (43, 46)	89
False Negatives	3 (2, 4)	6 (4, 8)	(85, 92)
False Positives	10 (10, 20)	10 (10, 19)	11 (8, 15)
True Negatives	965 (956, 965)	941 (931, 941)	9 (9, 18)
			891 (882, 891)

Karen R Steingart, et al. Cochrane Database Syst Rev. 2014 Jan 21;1

Prevalence (PV) depends on the specimen (form of TB)

TB forms	PV	PPV	NPV
Pulmonary smear-positive NTM infection also smear-positive	98% to 100%	98% to 99.5%	90%
Pulmonary smear-negative in occidental countries endemic countries screened with XRay or other test	2 – 5% 10% 30%	34 to 57%	97 to 99%
Extra-pulmonary specimen as CSF even if screened on the basis of Leucocytes > 10/mm ³	0.5%	8%	99.7%

Tricky to test extra-pulmonary smear-negative specimens

Prevalence (PV) depends on the specimen (form of TB)

TB forms	PV	PPV	NPV
Pulmonary smear-positive NTM infection also smear-positive	98% to 100%	98% to 99.5%	90%
Pulmonary smear-negative in occidental countries endemic countries screened with XRay or other test	2 – 5% 10% 30%	34 to 57%	97 to 99%
Extra-pulmonary specimen as CSF Even if screened on the basis of Leucocytes > 10/mm ³	0.5%	8%	99.7%

All smear-positive specimens can/should be tested for PCR

Molecular detection of Multidrug resistant-TB cases

Laboratories should aim to identify TB and rifampicin resistance in over 90% of cases **directly from smear + sputum** where resources are available for this...

... rapidly within 1-2 days

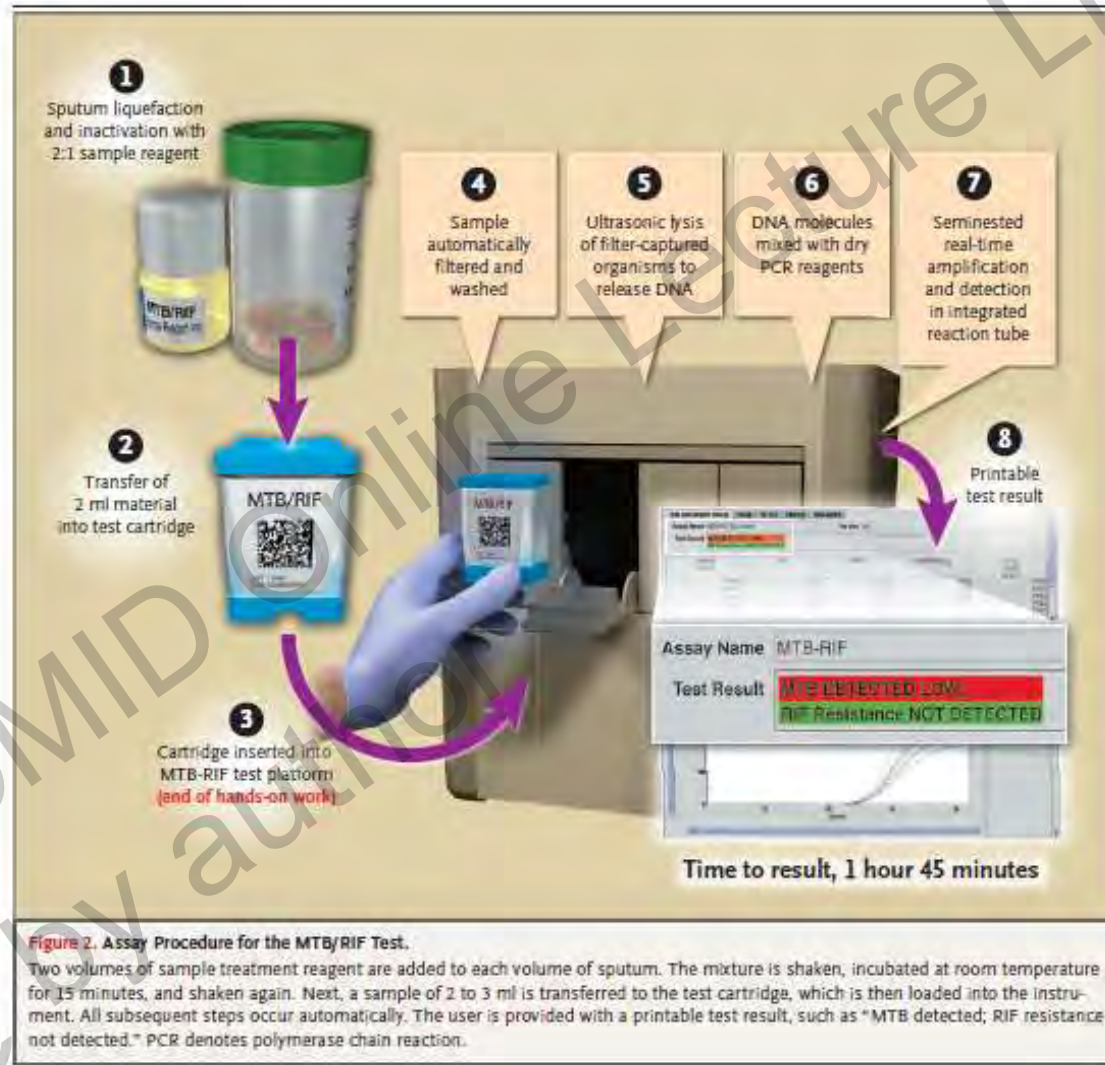
Eur Respir J 2006; 28: 1-7
DOI: 10.1183/09031936.06.00084906
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Recommended standards for modern tuberculosis laboratory services in Europe

F.A. Drobniowski*, S. Hoffner[#], S. Rusch-Gerdes[†], G. Skenders⁺,
V. Thomsen⁵ and the WHO European Laboratory Strengthening Task Force



Gene Xpert[®] MTB/RIF Cepheid (USA)

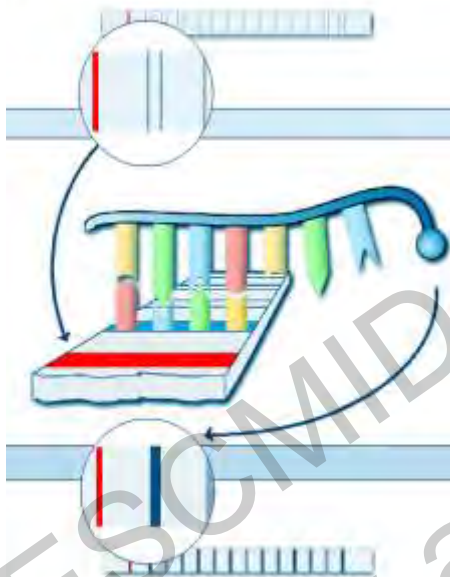


Boehme CC et al.
NEJM 2010

Genotype MTBDR_{plus}[®]

HAIN Lifescience
(Germany)

DNA-STRIP[®] with specific probes



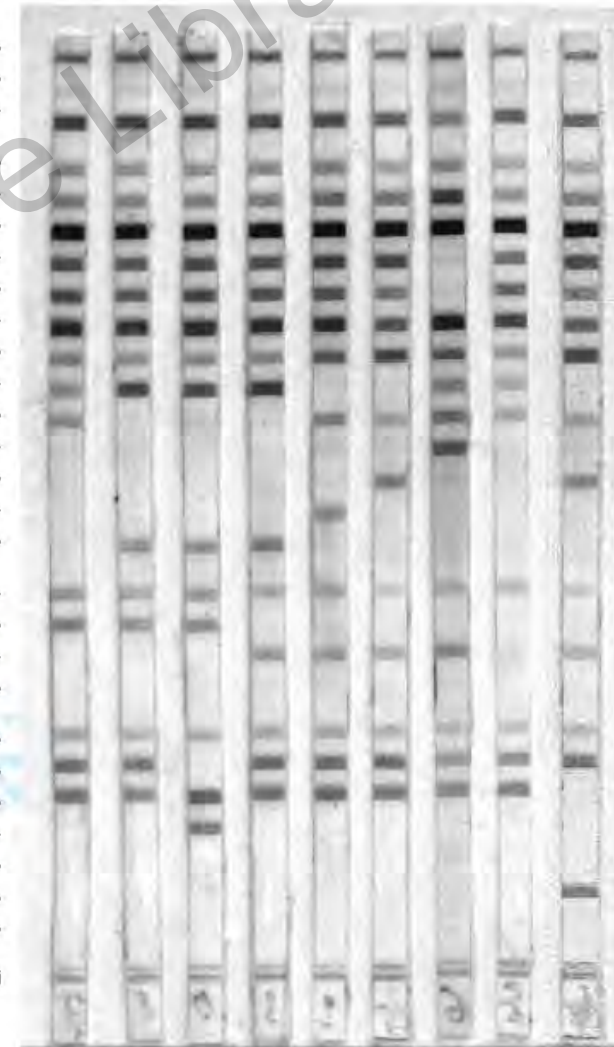
DNA-STRIP[®] with ensuing colour formation

Rifampicin
(*rpoB*-gene)

Isoniazid
(*katG*-gene)

Isoniazid
(*inhA*-regulatory region)

- CC -----
- AC -----
- Control Mtb -----
- Control *rpoB* -----
- rpoB* WT1 (506-509) -----
- rpoB* WT2 (510-513) -----
- rpoB* WT3 (513-517) -----
- rpoB* WT4 (516-519) -----
- rpoB* WT5 (518-522) -----
- rpoB* WT6 (521-525) -----
- rpoB* WT7 (526-529) -----
- rpoB* WT8 (530-533) -----
- rpoB* MUT1 (D516V) -----
- rpoB* MUT2A (H526Y) -----
- rpoB* MUT2B (H526D) -----
- rpoB* MUT3 (S531L) -----
- Control *katG* -----
- katG* WT (315) -----
- katG* MUT1 (S315T1) -----
- katG* MUT2 (S315T2) -----
- Control *inh* -----
- inh* WT1 (-16/-15) -----
- inh* WT2 (-8) -----
- inh* MUT1 (c15t) -----
- inh* MUT2 (a16g) -----
- inh* MUT3A (t8c) -----
- inh* MUT3B (t8a) -----
- CM



Molecular detection of rifampicin resistance

Sensitivity = all Resistant strains are detected
Specificity = no Susceptible strains are detected

- Cochrane review 2014 (prevalence 19%)
 - sensitivity : **95%** (95% CrI 90% to 97%)
 - specificity : **98%** (95% CrI 97% to 99%)
- WHO study group review
 - sensitivity : **95%** (95%CrI 90-97%)
 - specificity : **98%** (95%CrI 97-99%)

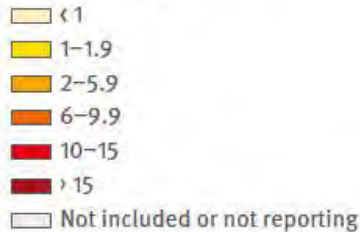
Positive predictive value for detection of rifampicin resistance

For 1000 patients, sensitivity of 95% and specificity of 98%

Prevalence of resistance	30%	15%	5%	2%
Nb of resistant isolates	300	150	50	20
Nb of resistant detected	285	143	48	19
Nb of resistant missed	15	7	2	1
Nb of false resistant test	14	17	19	20
PPV of detection of rifampicin resistance	96%	89%	72%	49%

MDR rates among TB cases in EU

Map 8: Percentage of notified TB cases with multidrug resistance among all TB cases with DST results, European Region, 2013



**4.1% overall,
2.6% in new cases pulmonary TB
17.0% in previously treated cases**

- Andorra
- Liechtenstein
- Luxembourg
- Malta
- Monaco
- San Marino

Positive predictive value for detection of rifampicin resistance

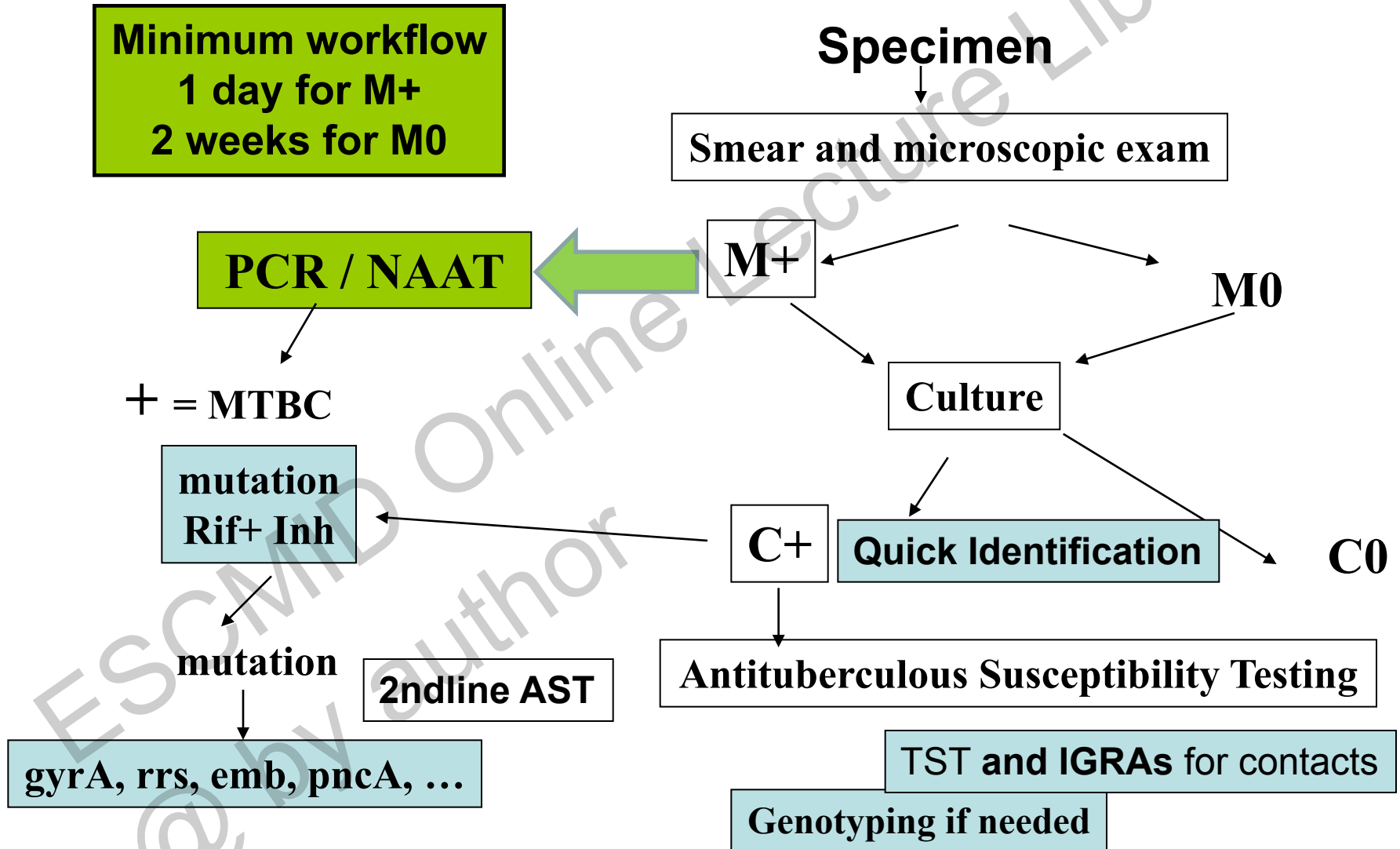
For 1000 patients, sensitivity of 95% and specificity of 98%

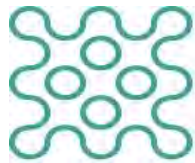
	Previously treated	New cases
Prevalence of resistance	15%	2%
Nb of resistant isolates	150	20
Nb of resistant detected	143	19
Nb of resistant missed	7	1
Nb of false resistant test	17	20

Confirmation with another molecular test and with phenotypic determination is mandatory

Integration of PCR in the new strategy for bacteriological diagnosis of tuberculosis

Minimum workflow
1 day for M+
2 weeks for M0





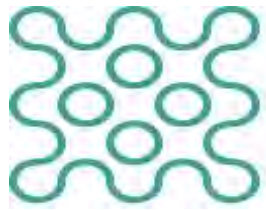
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More sessions on mycobacteria at ECCMID2015 Copenhagen

- **Sunday April 26th 7.45-8.45** Hall J: Meet the experts
How and when to treat patients with infections due to
Mycobacterium abscessus and other NTMs
- **Sunday April 26th 9 - 11** Hall J: Current topics in
tuberculosis
- **Sunday April 26th 11.30 -12.30** Hall I: Advances in
mycobacterial infections
- **Tuesday April 28th, 2013 11.30- 12.30** Hall K: OS35
Clinical aspects of mycobacterial infections
- **Poster sessions:** EV17, EP01, P29, P62, P63



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More information on ESGMYC

- **Website** : <http://www.escmid.org/esgmyc>
- **Meeting on Sunday April 26th in Meeting room 23**
- **18.15 to 19.15**
- **Want to become a member?**
 - logging to ESCMID
go to ESGMYC Study group
ask for membership
send a short CV and letter
 - You are accepted!!

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