



ESGMYC

ESCMID STUDY GROUP
FOR MYCOBACTERIAL
INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

New and old tools for tuberculosis diagnosis



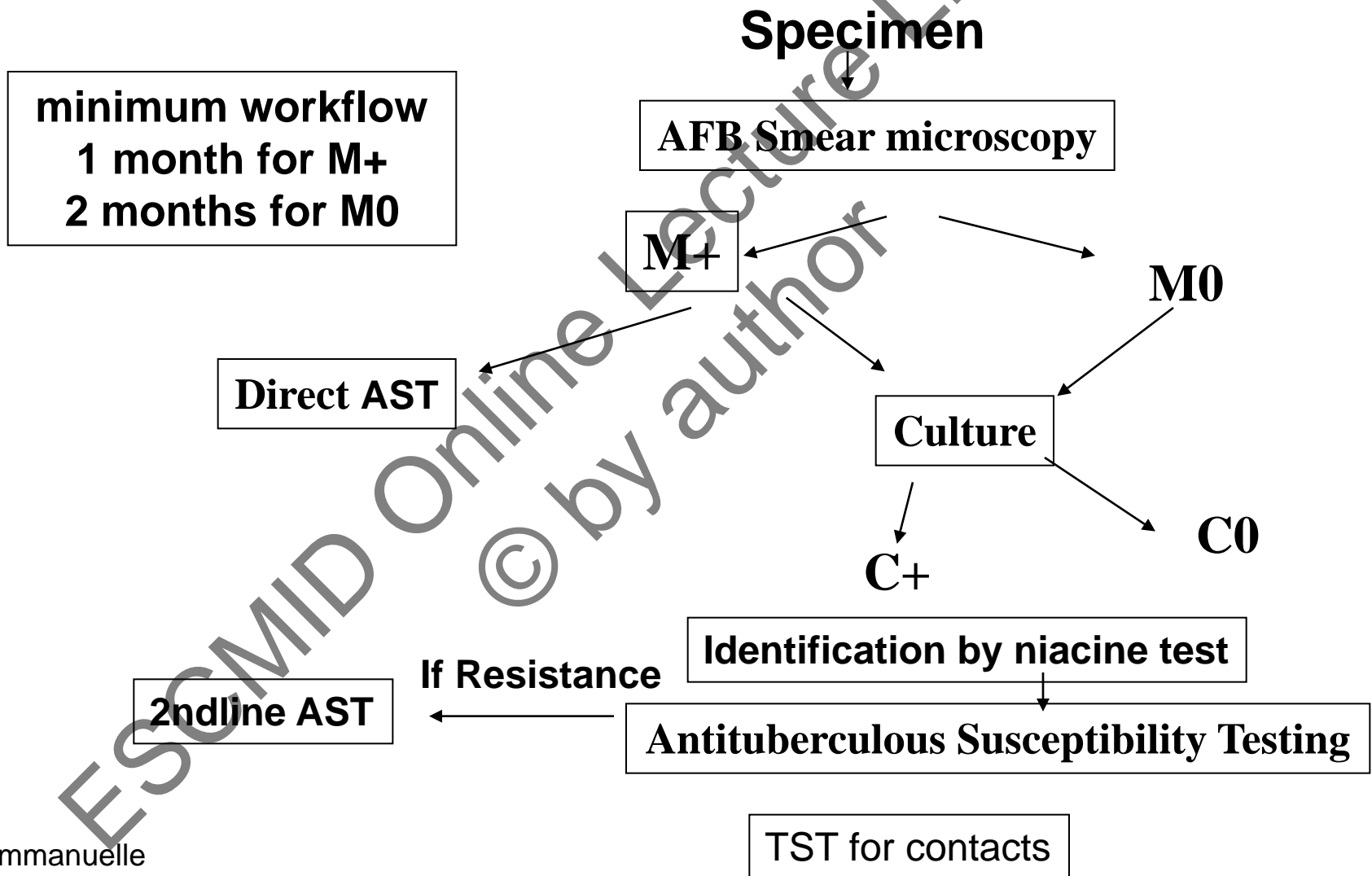
Emmanuelle CAMBAU
University Paris Diderot,
APHP, Saint Louis-Lariboisière Hospital,
NRC mycobacteria, Paris, France



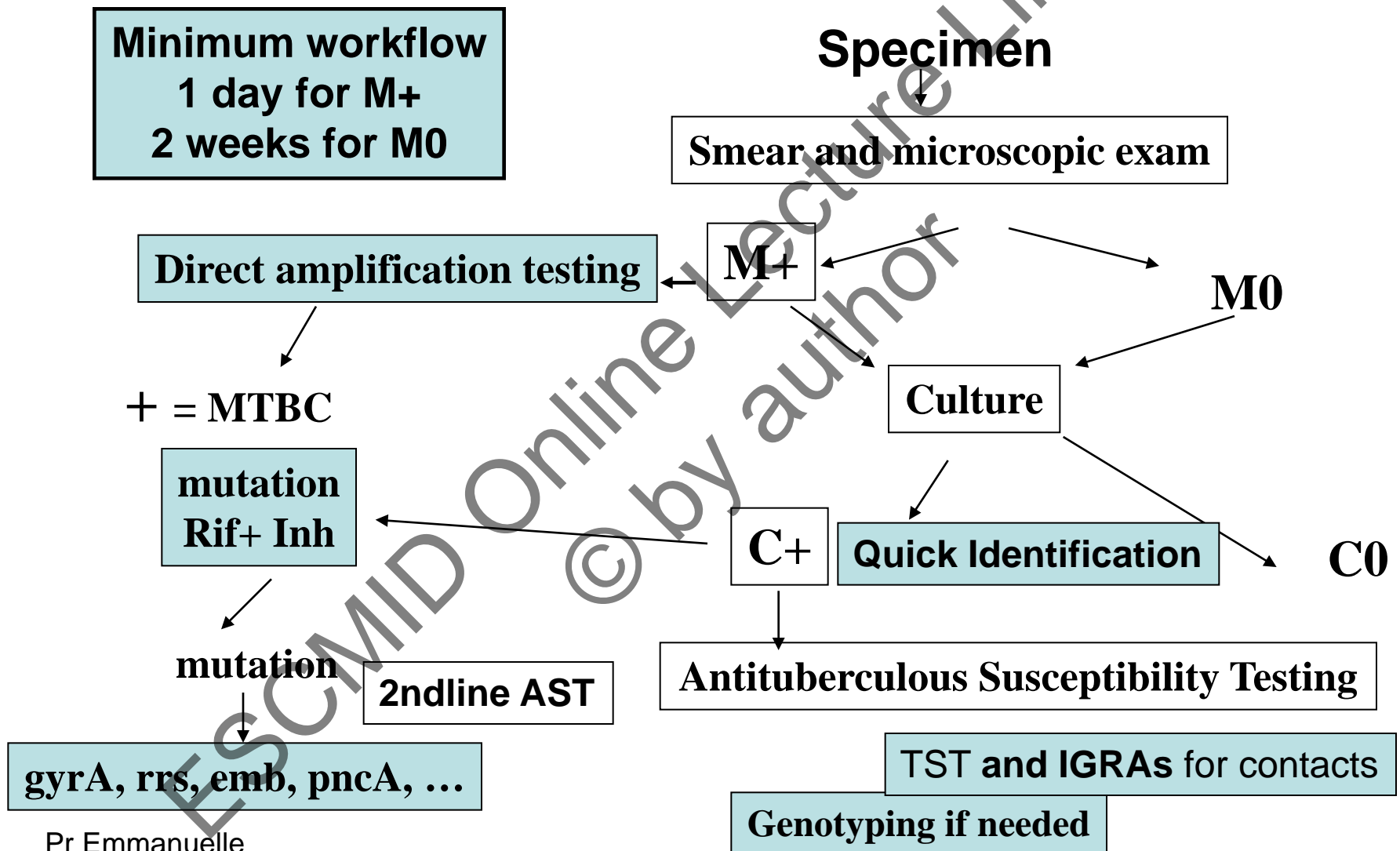
<http://cnrmymbt.free.fr/>

Educational Workshop - ECCMID 2012 London
the basis in tuberculosis diagnosis and management of cases

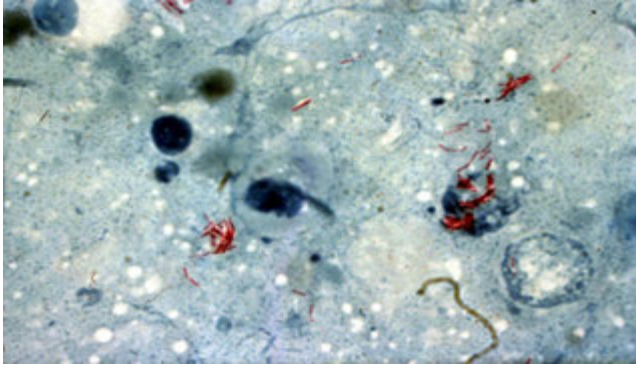
Strategy for bacteriological diagnosis of tuberculosis – Old tools



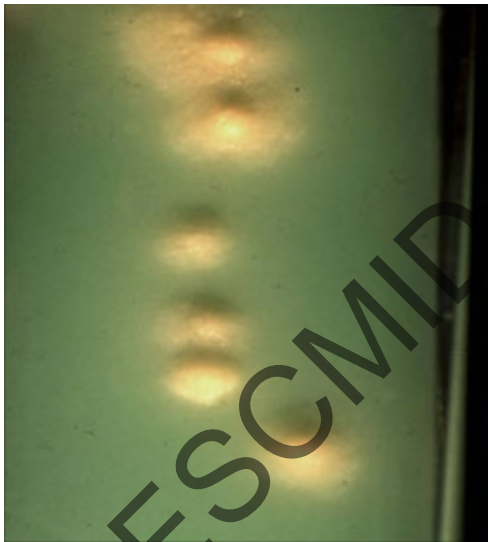
Strategy for bacteriological diagnosis of tuberculosis – New tools



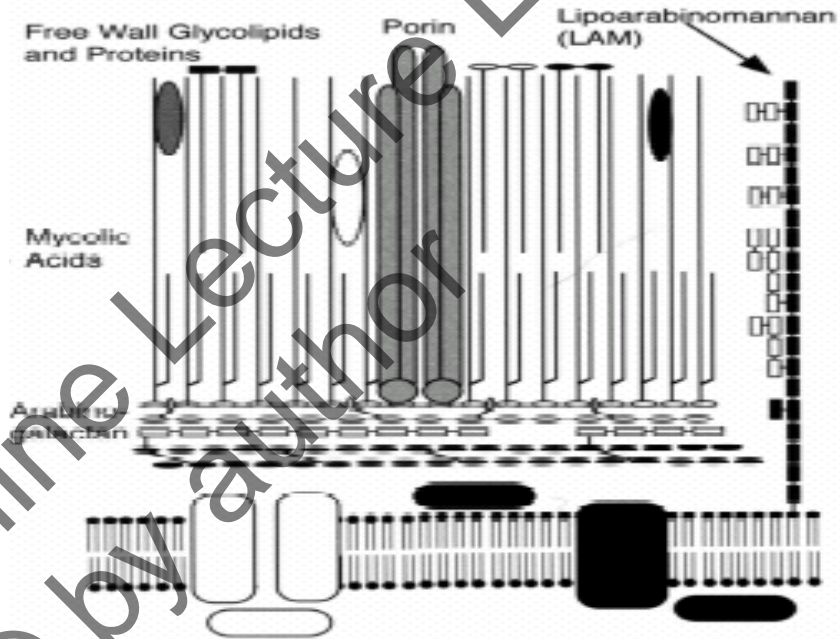
Specificities of tuberculous bacilli (*M. tuberculosis* complex)



Acid-fast bacilli (Ziehl-Neelsen)



Slow growth on rich medium
(e.g. Löwenstein-Jensen)



Lipid rich cell wall
(mycolic acids C60-C90)
⇒ Acids and NaOH resistance
⇒ Antibiotic intrinsic resistance

Cell Wall and cytoplasmic membrane
Cyo



Cultures of *Mycobacterium tuberculosis* complex

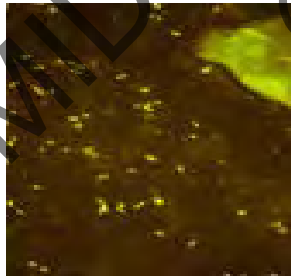
Require biosafety level 3 laboratory (BSL3)

- safety measures
- safety cabinets
- personal protection



Acid Fast bacilli smear examination

- Appropriate specimen
- Microscopy + Light-emitting diodes (LED)
- Auramine staining + automated stainers
- Ziehl Neelsen staining + automated stainers
- Sensitivity of 10,000 to 100,000 bacilli per ml



Reporting the smear results

Table 4.2. Reporting of microscopy smears

IUATLD/WHO scale (1000x field = HPF)	Microscopy system		
	Bright field (1000x magnification: 1 length = 2 cm = 100 HPF)	Fluorescence (200–250x magnification: 1 length = 30 fields = 300 HPF)	Fluorescence (400x magnification: 1 length = 40 fields = 200 HPF)
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty	1–9 AFB/1 length or 100 HPF	1–29 AFB/1 length	1–19 AFB/1 length
1+	10–99 AFB/1 length or 100 HPF	30–299 AFB/1 length	20–199 AFB/1 length
2+	1–10 AFB/1 HPF on average	10–100 AFB/1 field on average	5–50 AFB/1 field on average
3+	>10 AFB/1 HPF on average	>100 AFB/1 field on average	>50 AFB/1 field on average

From ECDC laboratory Handbook: <http://ecdc.europa.eu>

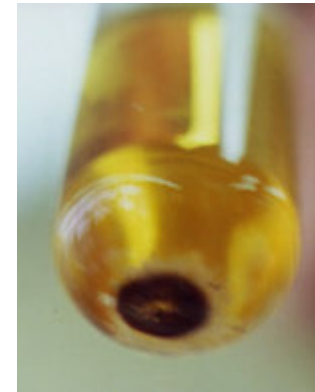
Primary culturing

Media	Smear pos	Smear neg
Solid LJ, Coletsos, Middlebrook	14 - 21 days	21- 42 days
Liquid* +automated systems	5 - 10 days	10 - 28 days

* Rapid detection but often required 1-2 supplementary days or secondary culturing for ID and AST testing



CAMBAU



Identification at the *M. tuberculosis* complex

- DNA / RNA Hybridization

Ex. ACCUPROBE *M. tuberculosis* complex (GenProbe, CA, USA)

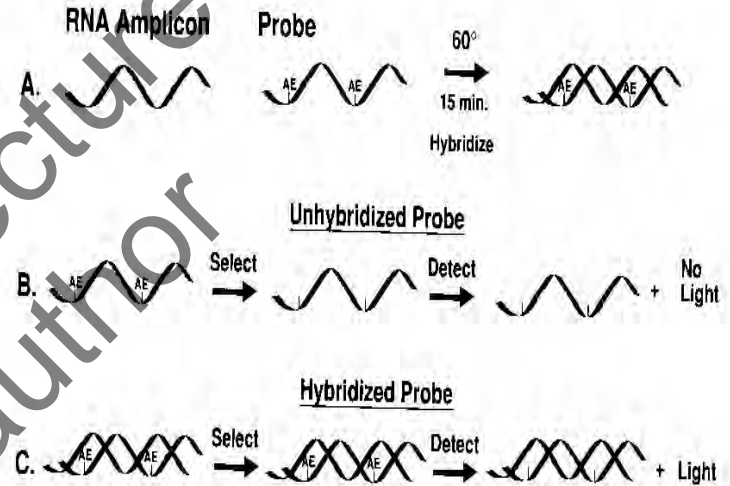
- Nucleic acid amplification

AND

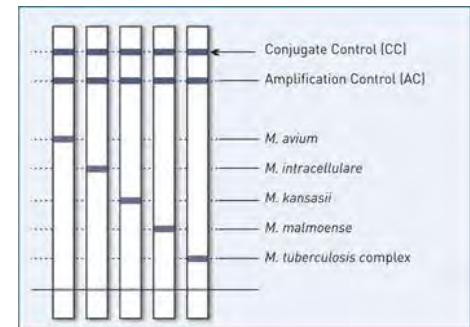
- ex hybridization

Ex. INNOLiPA Mycobacteria (Innogenetics)

GenoType Mycobacteria (Hain)

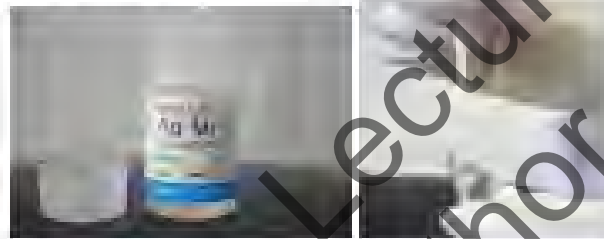
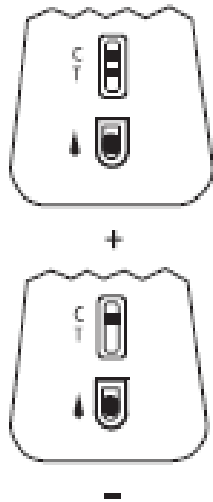


Marker line	Species
Conjugate control	1
MTB genes	3
MTB complex	3
WVA-1	4
WVA-2	5
WVA-3	6
HKE	7
MGD	8
MGV	9
MSI	10
MMU	11
HCE	12
MAIS	13
MAV	14
MIN-1	15
MIN-2	16
HSC	17
MHL	18
MHP	19
MCH-1	20
MCH-2	21
MCH-3	22
MFD	23
MSM	24



Rapid Identification by immunochromatography

How to use



15 min incubation

NEGATIVE

POSITIVE

100µl of liquid culture

100µl of suspension of colonies

Control band only

Control band and
Additional band for
M. tuberculosis

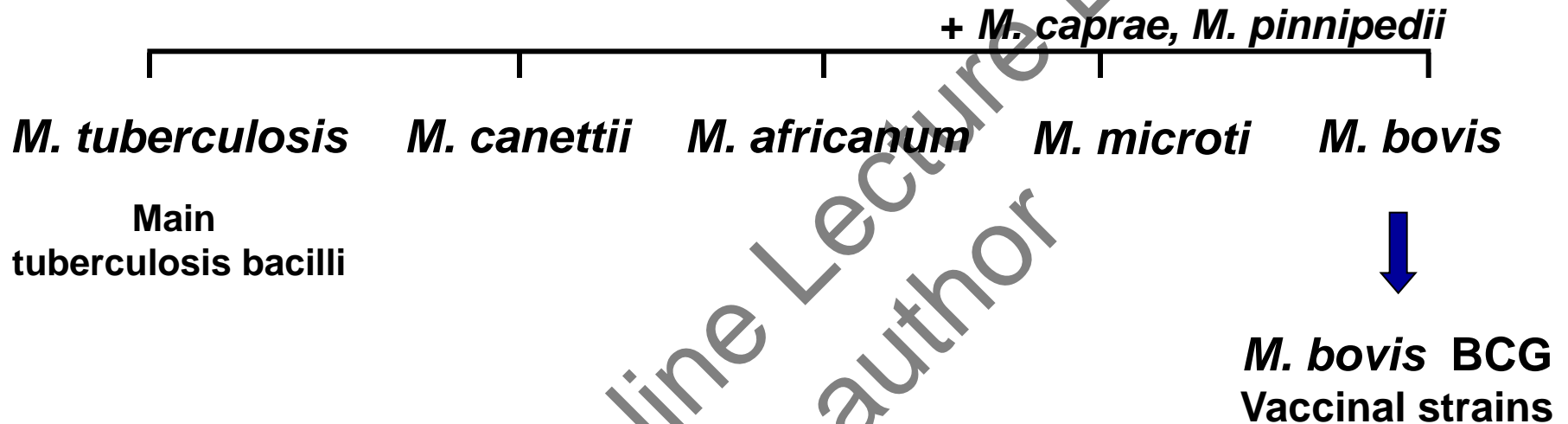
BD MGIT® TBc



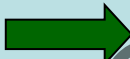
BIOLINE TB Ag. MPT64®

15 minutes, easy and cheap (5 euros)

Said JCM 2011

Mycobacterium tuberculosis complex



-  All are tuberculous bacilli
-  99 % DNA homology = one species
Common ancestor and deletions / subspecies
-  Identical 16S rRNA

Speciation within the *M. tuberculosis* complex

1. *M. tuberculosis*
or *M. canetti* or
M. africanum II

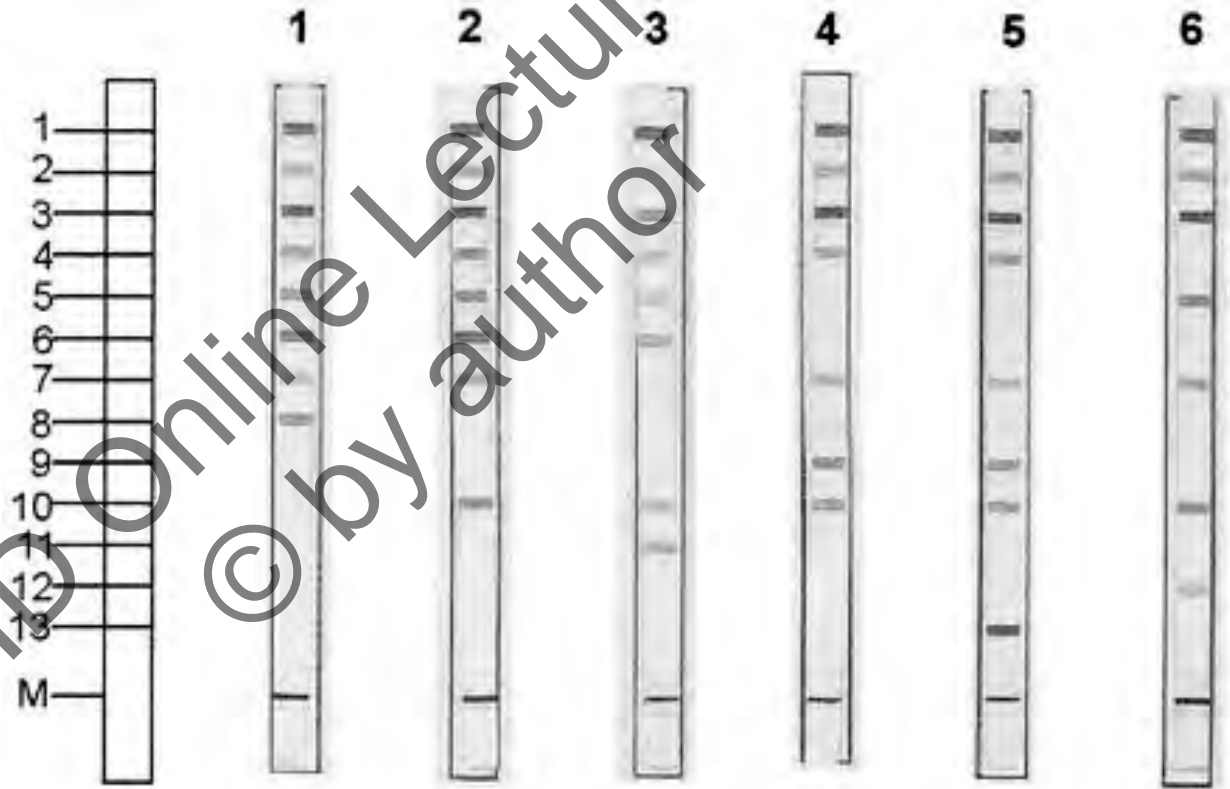
2. *M. africanum* I

3. *M. microti*

4. *M. bovis*

5. *M. bovis* BCG

6. *M. bovis caprae*



Serology for tuberculosis

- Meta-analysis : KR steingart et al. PLoS Medicine 2011
- Pulmonary (67 studies; 5,147 patients)
and extra pulmonary TB (25 studies; 1,809 patients)
 - Sensitivity : 0 to 100%
 - Specificity : 31-59% to 100%

Conclusions: Despite expansion of the literature since 2006, commercial serological tests continue to produce inconsistent and imprecise estimates of sensitivity and specificity. Quality of evidence remains very low. These data informed a recently published World Health Organization policy statement against serological tests.

Serology for tuberculosis

- example of best results -

Table 2. Bivariate meta-analyses: pooled sensitivity and specificity estimates by subgroup.

Subgroup	Number of Studies	Number of Participants (Sample Size)	Sensitivity	Specificity
Pulmonary TB				
anda-TB IgG, smear-positive	7	870 (870)	76 (63–87)	92 (74–98)
anda-TB IgG, smear-negative	4	700 (700)	59 (10–96)	91 (79–96)
ELISA	54	3,696 (6,434)	60 (6–65)	98 (96–99)
Immunochromatographic Test ^a	12	1,231 (1,512)	53 (42–64)	98 (94–99)
Extrapulmonary TB				
Lymph node TB	6	640 (922)	64 (28–92)	90 (76–97)
Pleural TB	5	322 (572)	46 (29–63)	87 (51–99)
anda-TB IgG	10	1,055 (1,637)	81 (49–97)	85 (77–92)

Sensitivity and specificity estimates given as posterior means (percent) with 95% credible intervals in parentheses.

^aSerological tests included: ICT TB (three studies), Assure TB (two studies), MycoDot (three studies), SDHO (two studies), Hexagon (one study), Serocheck-MTB (one study).

doi:10.1371/journal.pmed.1001062.t002



Drug susceptibility testing (DST) for *M. tuberculosis* complex



- Proportion method on solid or liquid medium
- Resistance Ratio method
- Absolute concentration method
- MIC microtitration
- Nitrate reductase
- Alamar Blue.....



4 to 12 weeks turn-around
time for results
from the day of sampling

Drobniewski, F. et al. CMI 2007, ECDC laboratory Handbook: <http://ecdc.europa.eu>

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

- PCR started in 1990 using
 - gene (Hermans JCM 1990)
 - RNA (Boddinghaus JCM 1990)
 - IS6110 (Thierry JCM 1990)
- 1996 : recommendations CDC / ATS
- 2008 : recommendations CDC / JAMA
- Pubmed
 - More than 4400 papers, 330 review, 6 meta-analyses
 - Sarmiento 2003: meta-analysis on smear negative specimens
 - Greco 2009: meta-analysis on smear positive specimens

ATS 1997, Ieven and Goosens 1997, Sarmiento 2003,
Dinnes 2007, MMWR 2009;58-7-10

Sensitivity of NAAT

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

FREDERICK S. NOLTE,^{1,2,3*} BEVERLY METCHOCK,^{3,4} JOHN E. MCGOWAN, JR.,^{3,4} ALISE EDWARDS,²
OGI OKWUMABUA,^{2,3} CATHY THURMOND,² P. SHAWN MITCHELL,² BONNIE PLIKAYTIS,⁵
AND THOMAS SHINNICK⁵

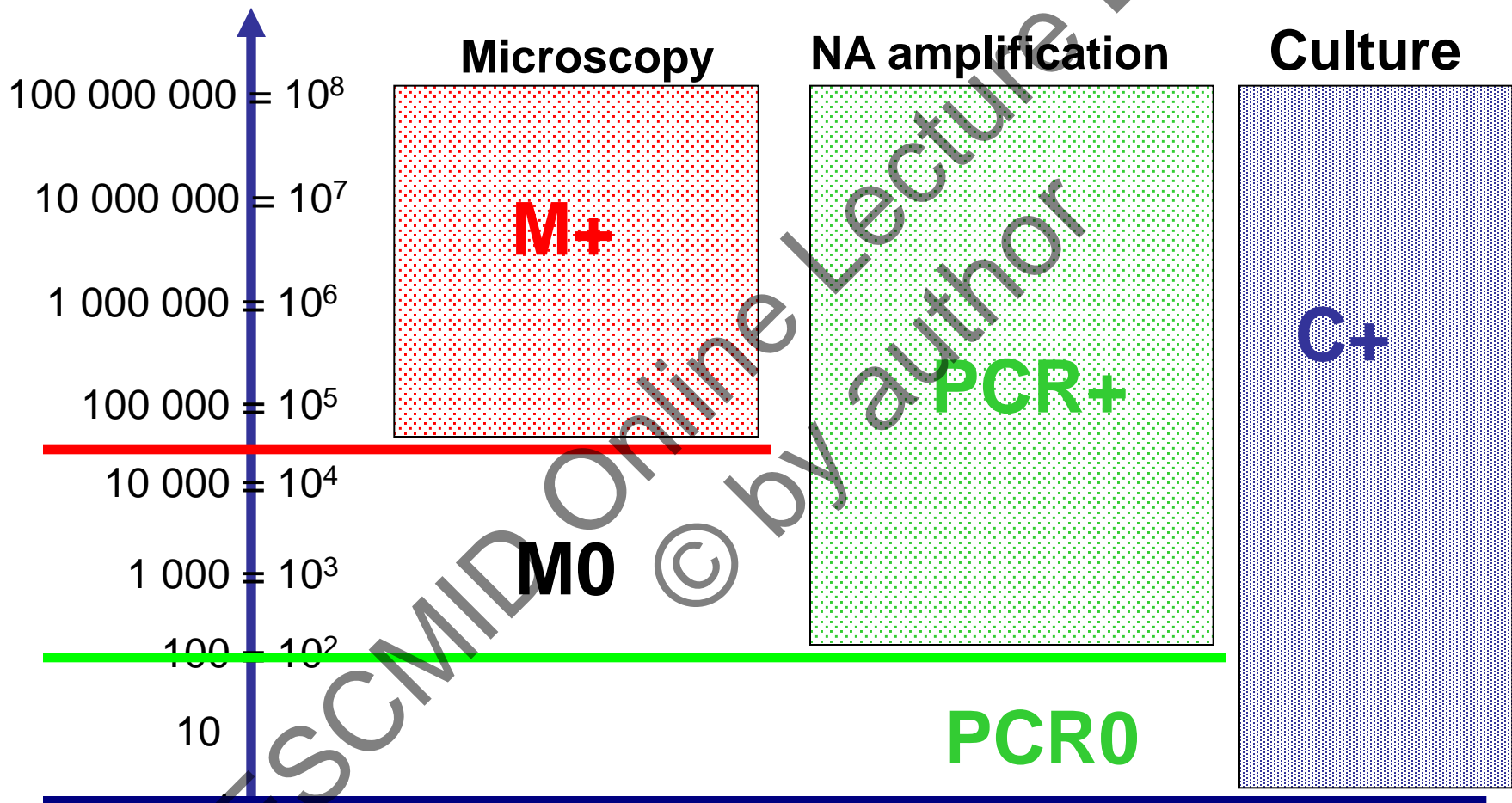
Emory University Hospital,¹ The Emory Clinic,² and Department of Pathology and Laboratory Medicine,
Emory University School of Medicine,³ Atlanta, Georgia 30322; Grady Memorial Hospital, Atlanta,
Georgia 30355⁴; and Hansen's Disease Laboratory, Division of Bacterial Diseases,
Centers for Disease Control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

Sensitivity of diagnosis tools for tuberculosis

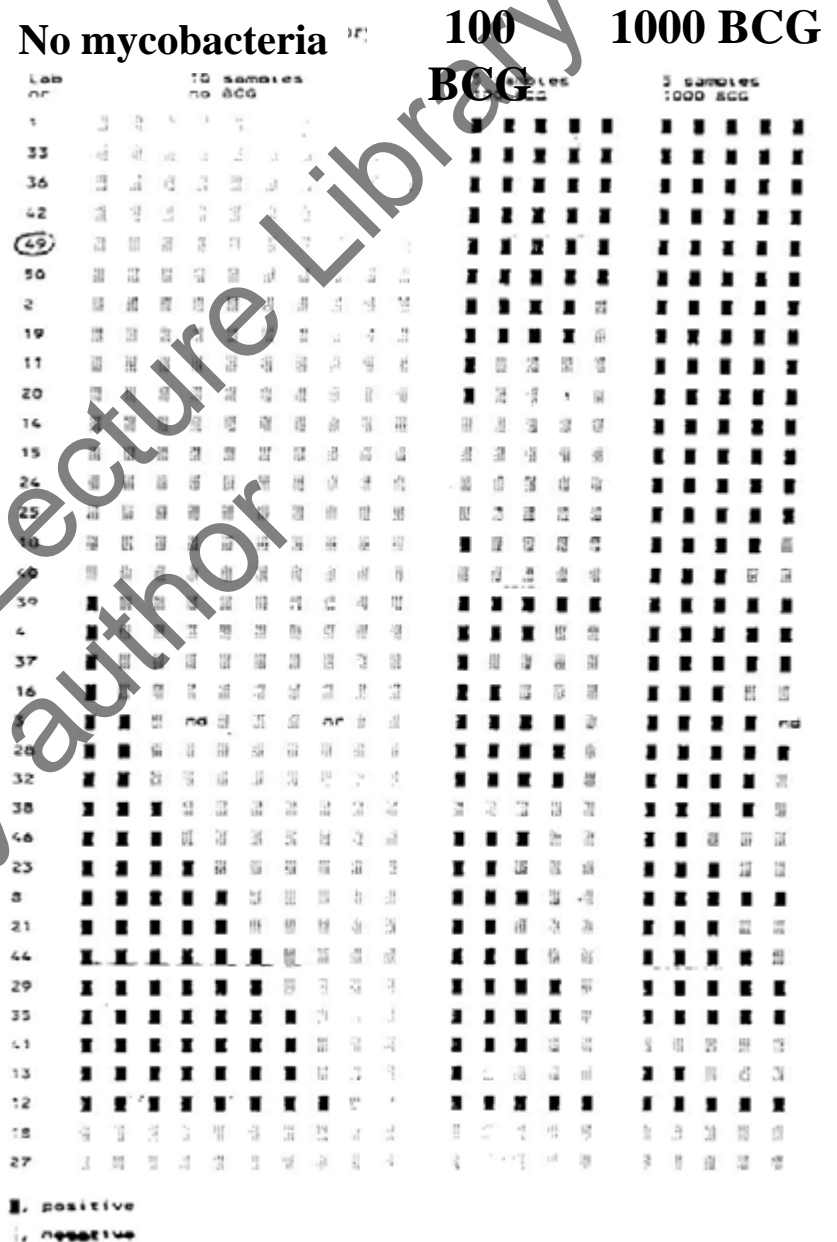
N per ml specimen



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 Performances for TB: specificity

(1) We would like that only TB patients are NAAT+

- Suspicion of smear positive pulmonary TB
 - Specificity = 98%
- Suspicion of smear negative pulmonary TB
 - Specificity = 86%
- Suspicion of extra-pulmonary TB
 - Specificity = 74.5% (62% on CSF)
- 11 studies with Xpert® MTB/RIF®
 - Specificity = 98.6%

Laraque, CID 2009; Helb 2010; Boehme 2010 and 2011; Rachow 2011;
Marlowe 2011; Armand 2011; Theron 2011 ; Bowles 2011; Scott 2011;
Miller, 2011; Teo, 2011

NAAT Performances for TB: sensitivity

(2) We would like that TB patients with smear negative specimens are NAAT+

- Sarmiento 2003: meta-analysis for smear-negative TB cases
 - « Sensitivity ranged from 9 to 100% »
 - « Specificity ranged 25 to 100% »
 - => Low positive predictive values
- 11 studies with Xpert®MTB/RIF®
 - Range: 47% - 83%

Helb 2010; Boehme 2010 and 2011; Rachow 2011; Marlowe 2011; Armand 2011
Theron 2011 ; Bowles 2011; Scott 2011; Miller, 2011; Teo, 2011

Indications of NAAT and tuberculosis

- Suspicion of pulmonary tuberculosis
 - Not for extra pulmonary TB (except if smear positive?)
 - Not for sequellae
 - Not for latent TB
 - Not for mycobacteriosis (except if smear positive?)
- All smear positive cases=> Positive diagnosis of TB
- Smear negative cases if highly suspicion of TB (prevalence > 5% => PPV > 50%)
=> early diagnosis of TB

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
Copyright © ERS Journals Ltd 2006

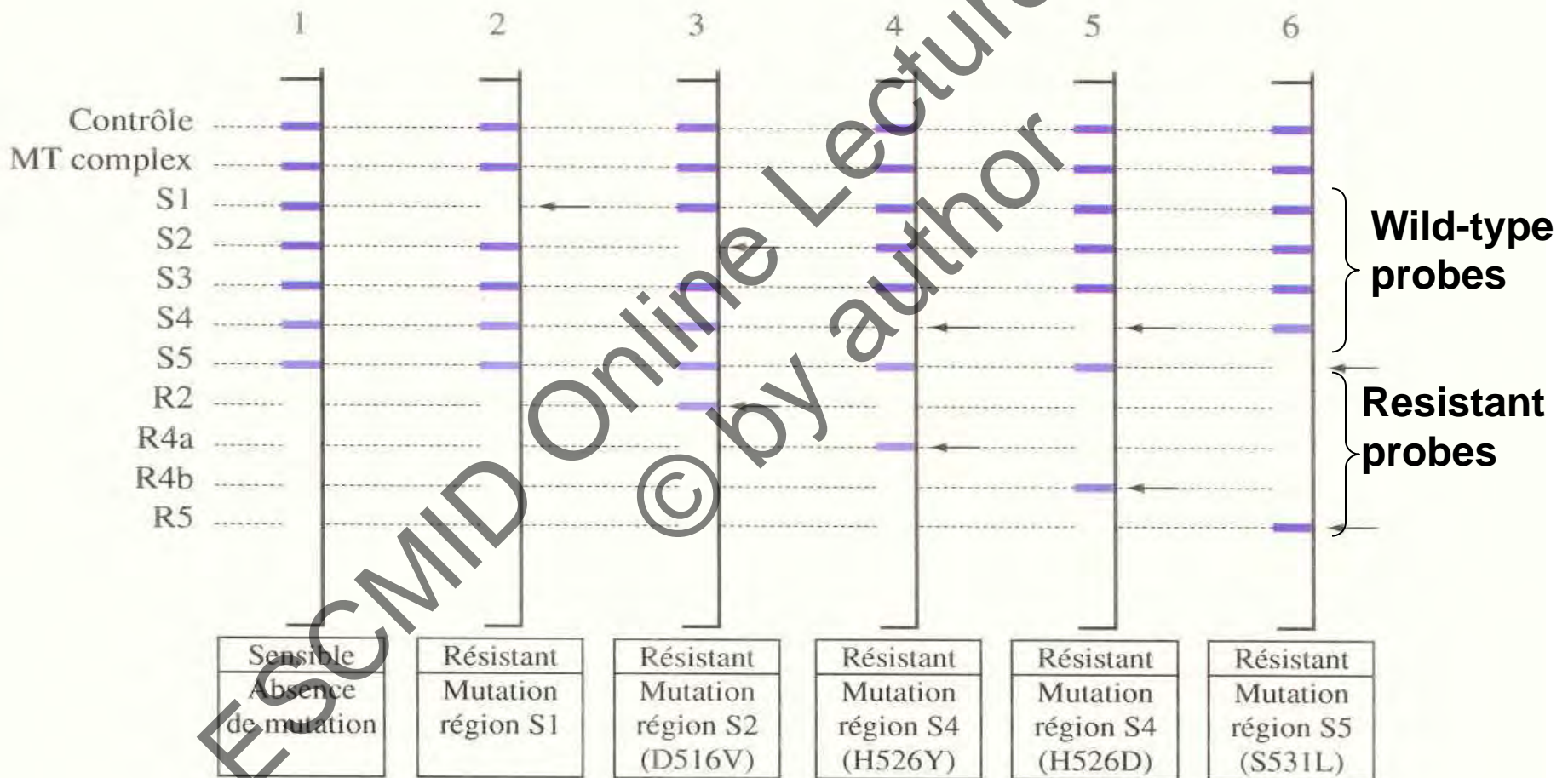
Recommended standards for modern tuberculosis laboratory services in Europe

F.A. Drobniowski[†], S. Hoffner[#], S. Rusch-Gerdes^{†1}, G. Skenders⁺,
V. Thomsen⁵ and the WHO European Laboratory Strengthening Task Force



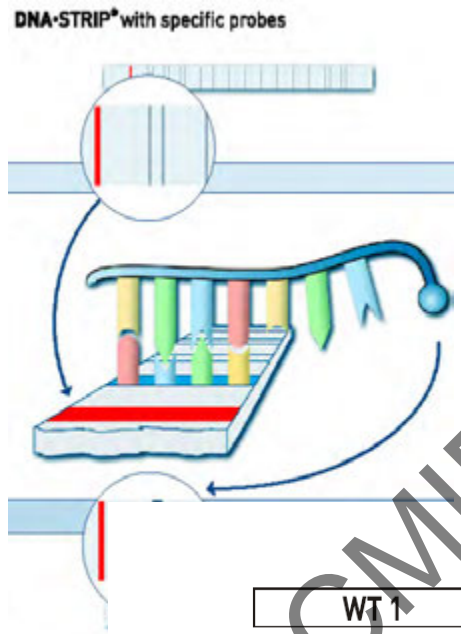
INNO-LiPA - Rif^rTB

Innogenetics (Belgium)



Genotype MTBDR_{plus}[®]

HAIN Lifescience
(Germany)

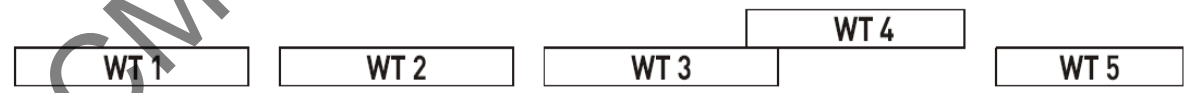
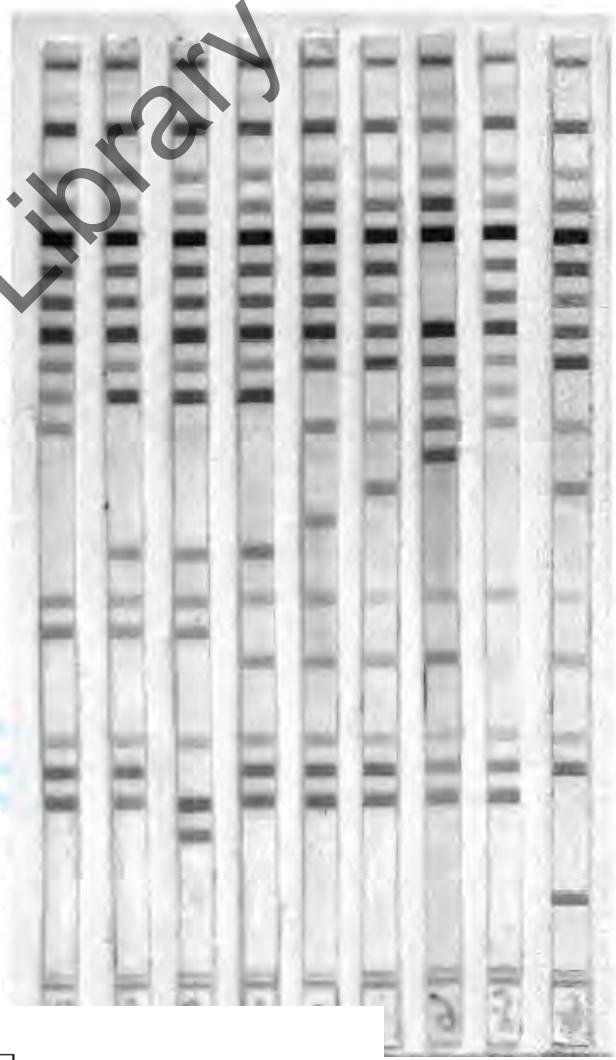


Rifampicin
(*rpoB*-gene)

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 (e15t) -----
- inh* MUT2 (a16g) -----
- inh* MUT3A (t8c) -----
- inh* MUT3B (t8a) -----
- CM -----



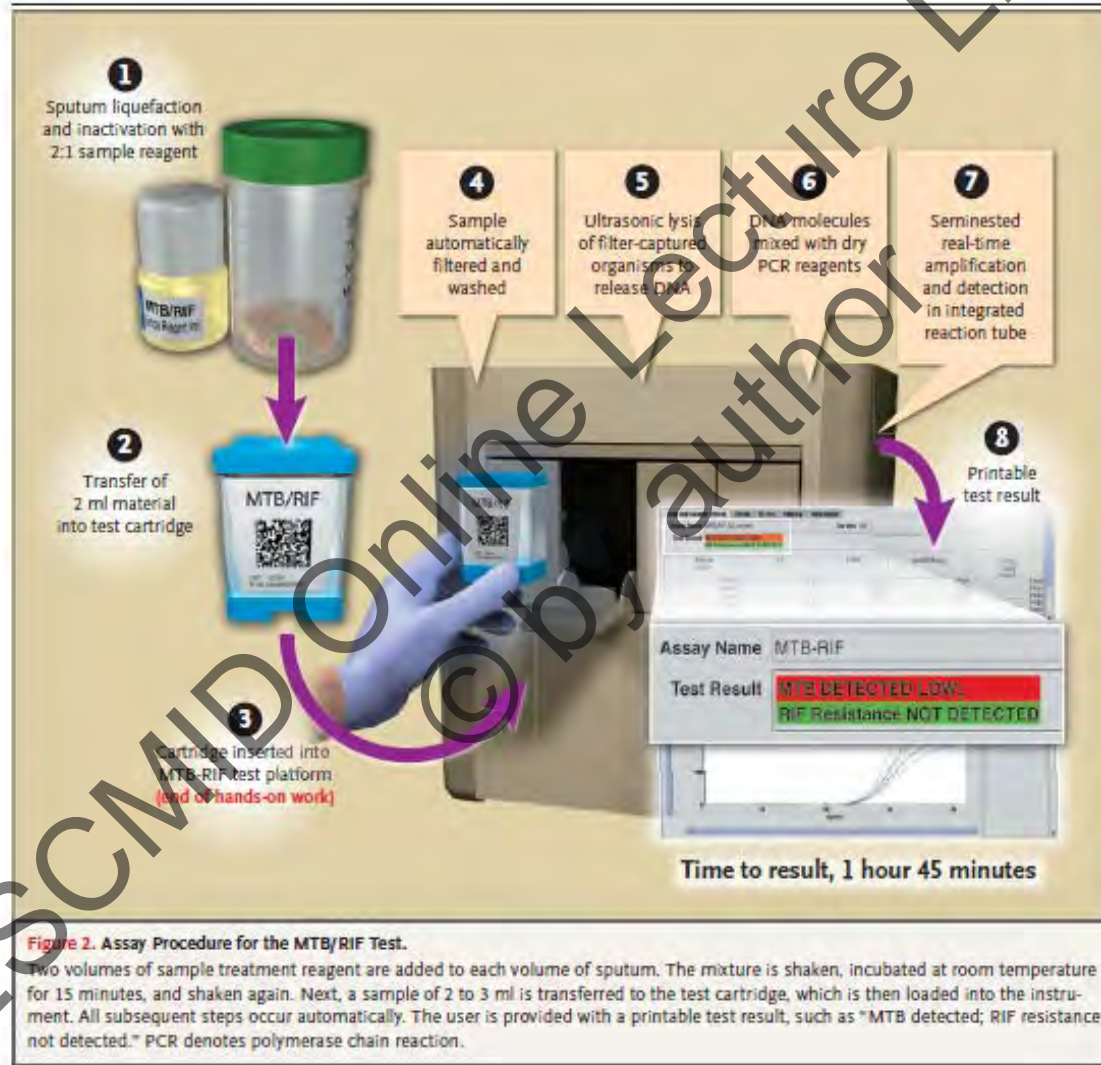
Pr Em
CAME...

MUT D516V

MUT H526Y
MUT H526D

MUT S531L

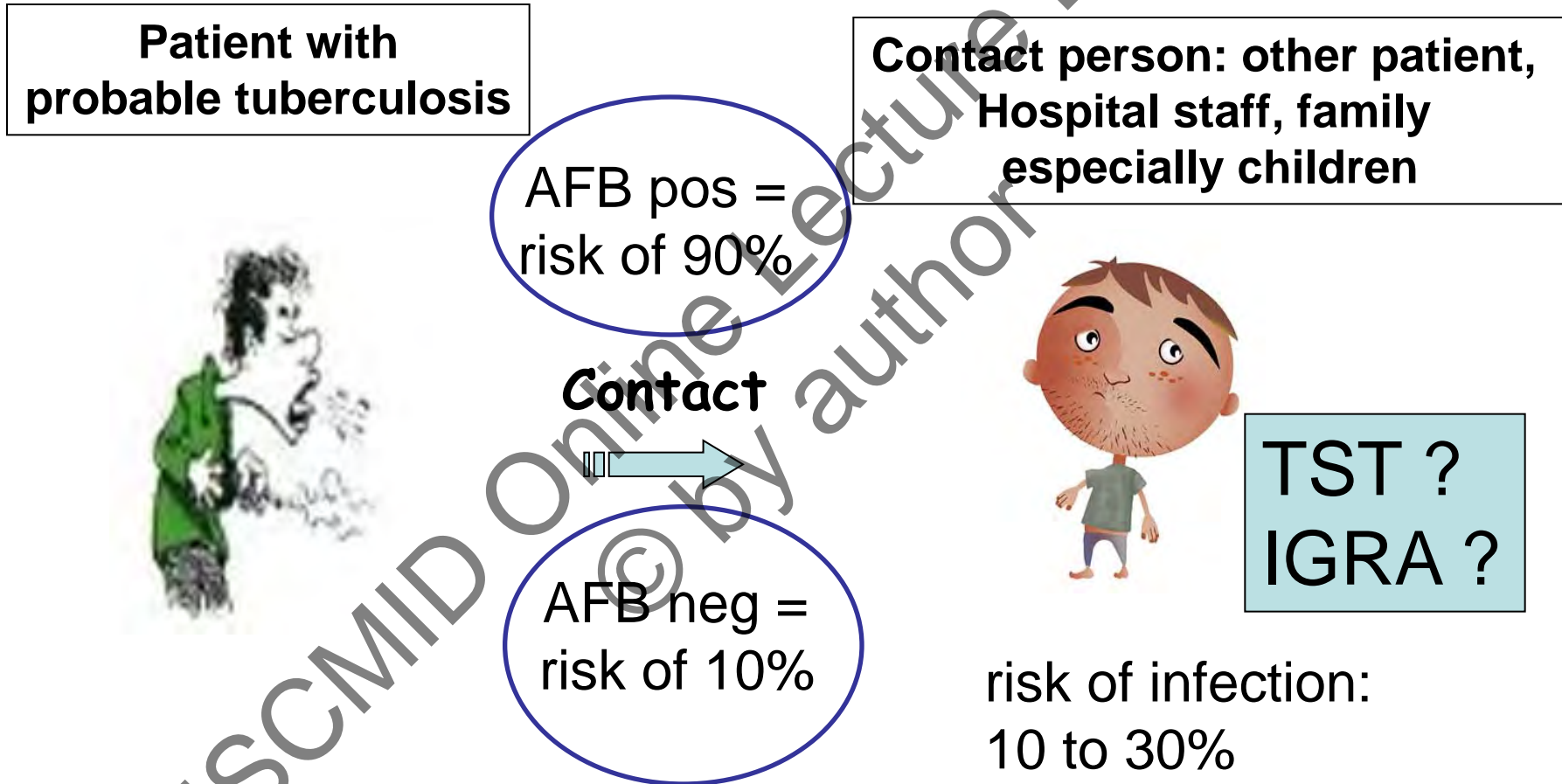
Gene Xpert[®] MTB/RIF Cepheid (USA)



Performances of molecular kits for direct detection of MDR-TB

- Identification of *M. tuberculosis* complex AFB
 - YES, all kits
 - 98 to 100% sensitivity on smear-positive respiratory specimens
- Detection of drug resistance associated mutations
 - *rpoB* (beta subunit of RNA polymerase) mutations associated with rifampicin resistance
 - Yes, all with a 95-100% sensitivity
 - Isoniazid resistance associated mutations in *katg* and *inhA* = GenoType[®] MTBDRplus

Transmission and latent TB

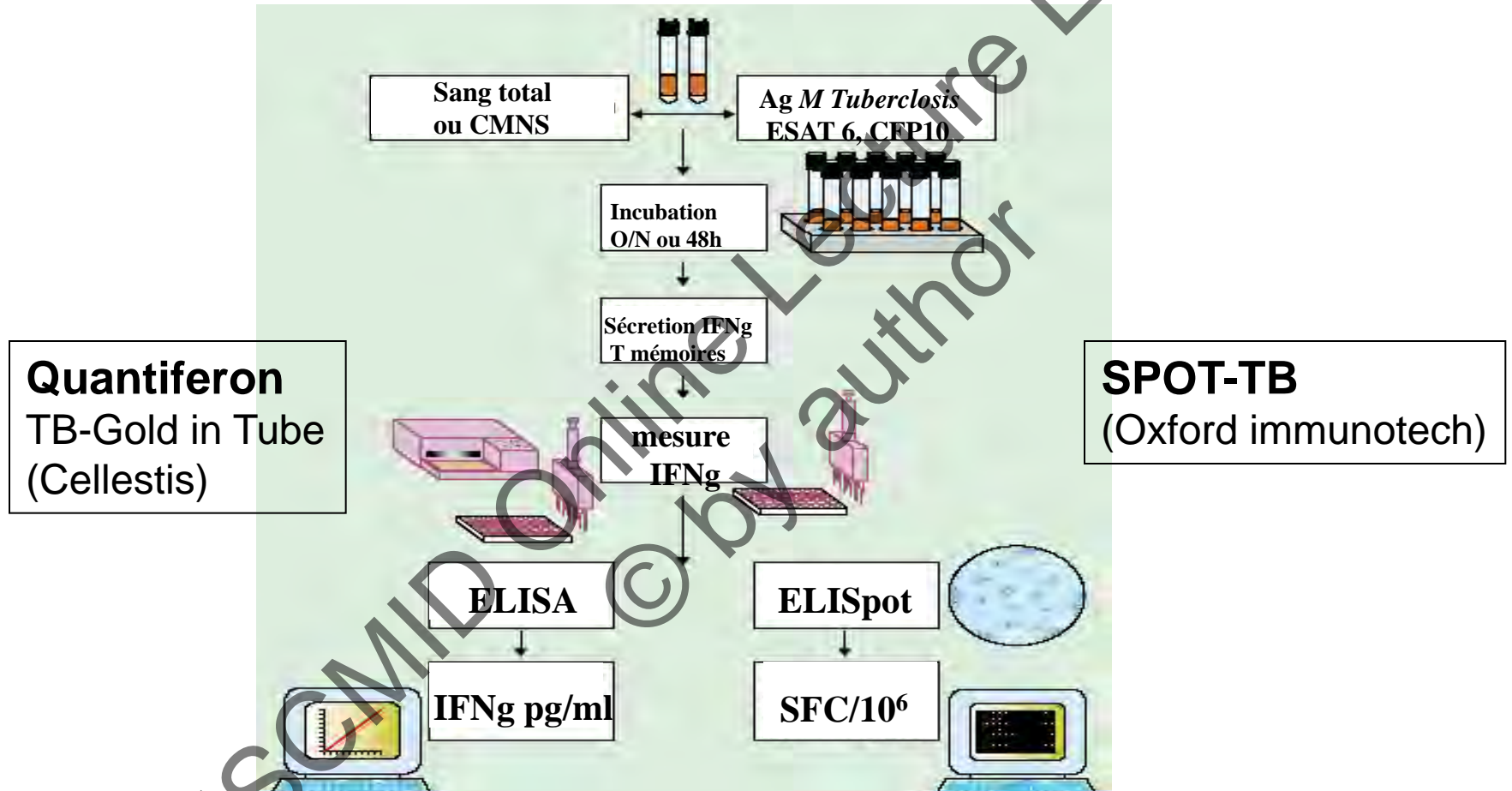


Interferon Gamma release Assays

- ECDC recommendations 2011
- 6 reviews and meta-analyses in 2011:
 - CID 2011: Herrera V. et al. 2011 (52:1031-1037)
 - JAIDS 2011: Cattamanichi A et al. (56:230-238)
 - Eur Respir 2011: Diel R. et al. (37:88-99)
 - CMI 2011: Denkinger CM et al. (17:806-814)
 - Curr Opin Rheumatol 2011: Smith R et al. (23:377-384)
 - JID 2011: Metcalfe JZ et al. (204:S1120-1129)

IGRA tests for TB

Production of IFN γ by T lymphocytes after stimulation with specific antigens ESAT-6, CFP-10 (TB7,7)



Quantiferon
TB-Gold in Tube
(Cellestis)

SPOT-TB
(Oxford immunotech)

= in vitro TST with TB specific antigens lacking in BCG strains

New tools versus Old tools conclusions

- Turn around times are shorter =>
 - Tuberculosis diagnosis is obtained earlier
- Main new tools
 - Molecular detection of resistance
 - Rapid identification of MTB cultures
- Still
 - Quality and expertise : Skilled technicians
 - Evaluation of your practices

Rush-Gerdes et al. 1999



ESGMYC

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INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

More information

- ESCMID group on mycobacterial infections (ESGMYC):
- **Meeting on Sunday April 1st in Meeting room 8
from 13.00 to 14.00**
- **Website : <http://www.escmid.org/esgmyc>**
- Surveillance reports for TB by the European Center for Disease Prevention and Control (ECDC) :
http://ecdc.europa.eu/en/publications/surveillance_reports/
- Laboratory Handbook from ECDC and European reference center network for Tb (ERLNet-TB)
« Mastering the basics of TB control »