

# Effective strategies for the diagnosis of multidrug resistant tuberculosis

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and their drug resistance



ESGMYC

ESCMID STUDY GROUP  
FOR MYCOBACTERIAL  
INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

## Diagnosis of MDR-TB: objectives

1. Detection of MDR-TB cases
2. Prevention of MDR-TB spread (no transmission to other persons)
3. Curing the patient (individual treatment management of the tuberculosis case)
4. Drug-resistance surveillance (scale of a hospital, city, region, country, world)

## Diagnosis of MDR-TB: today 's presentation

### **Curing the patient** : Individual treatment management of the tuberculosis case

- Detect resistance to all drugs
- Assess susceptibility to all non-R drugs
- Report results as soon as possible, especially if transmission might going on
- Give best effective treatment
- Prevent the selection of more resistant Mtb strains
  - for preventing secondary TDR resistance
  - for preventing increase in TDR primary resistance



## Cultures of *Mycobacterium tuberculosis* Complex

Require **biosafety level 3 laboratory (BSL3)**

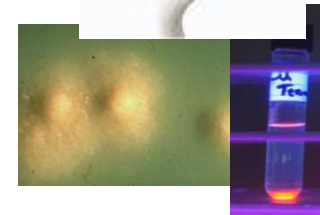
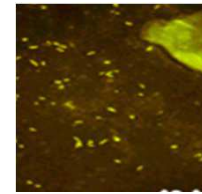
- safety measures
- safety cabinets
- personal protection



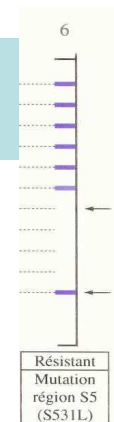
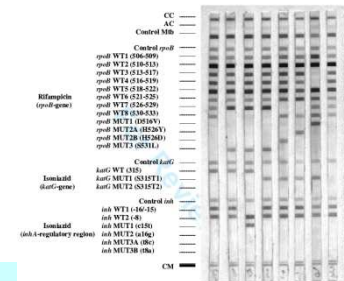
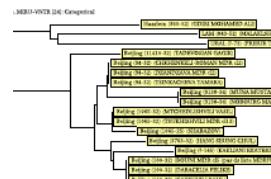
**Mandatory for  
MDR-TB strains**

# Tools for the bacteriological diagnosis of MDR-TB

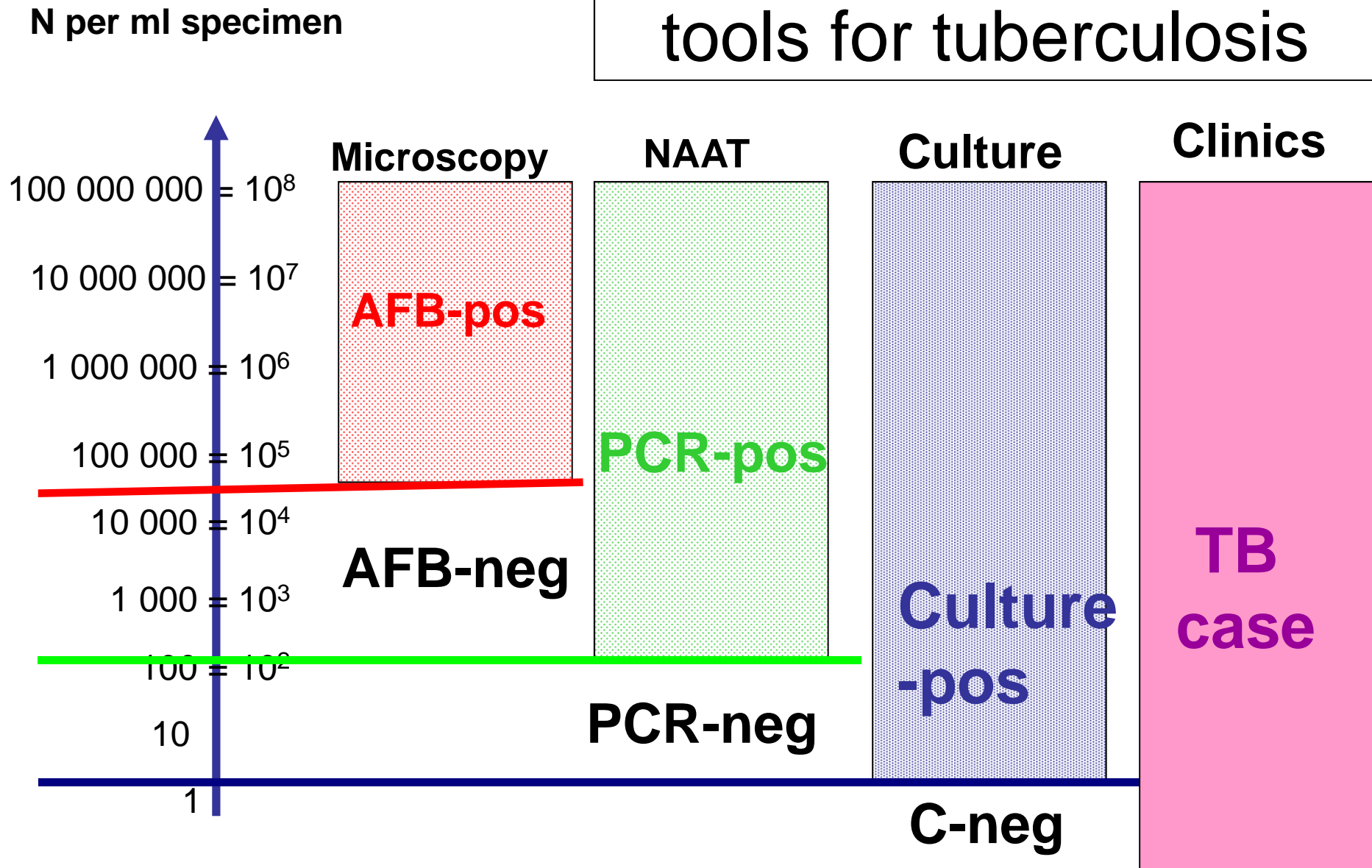
- Smear microscopy of respiratory specimens (ME)
- Direct nucleic acid amplification (NAAT/ PCR)
- Culture (liquid and solid media) and identification
- Drug (antibiotic) susceptibility testing (DST)
- Molecular detection of resistance
- Genotyping



**H R E S +  
Z Eth C FQ K Akn Pas Lnz**

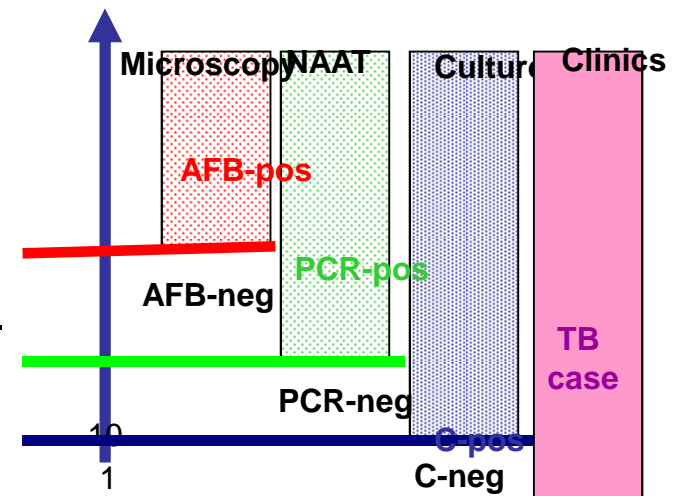


# Sensitivity of diagnosis tools for tuberculosis



## Low sensitivity of PCR in smear-negative specimens

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



**Needs also diagnosis tool for culture-negative TB cases**

Steingart et al. Cochrane Database Syst Rev. 2013 and 2014,  
LID review (Lawn S et al. 2013); WHO publications 2014 <http://www.who.int/tb/>



Low positive predictive value in extra-pulmonary TB because of 98-99% specificity

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

Tricky to test extra-pulmonary smear-negative specimens



# False detection of resistance where resistance prevalence is low

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

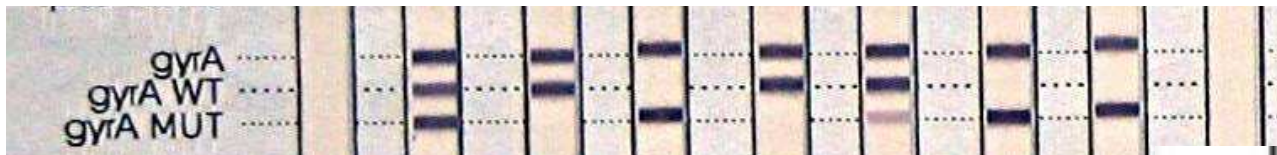
Prevalence of resistance	30%	2%
Nb of resistant isolates	300	20
Nb of false resistant test	14	20
PPV of detection of rifampicin resistance	96%	49%

**Confirmation with another molecular test and with phenotypic determination is mandatory in countries with prevalence MDR < 20-30%**

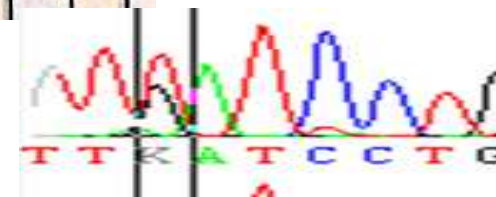
Karen R Steingart<sup>1</sup>, et al. Cochrane Database Syst Rev. 2013

# Being able to detect heteroresistance

- **heteroresistance = not 100% resistant mutant**
- Genotypic methods less sensitive than phenotypic methods (1% proportion)
  - wild type allele if resistant mutant < 10%
  - Mixed genotypes between 10% and 100%
- DNA strip methods: wt and mut bands

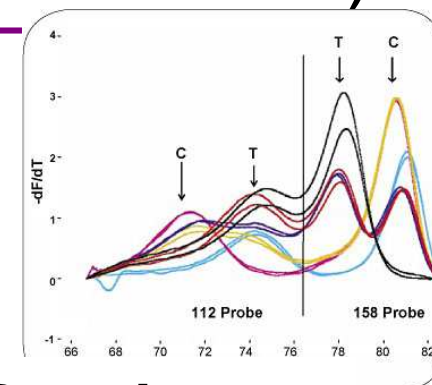


- Sequencing: two peaks
- WGS: 10X to 100X coverage is necessary



# Commercial tests for molecular detection of resistance to all anti-TB drugs (resistome)

Antibiotic	Main genes involved
Rifampicin	rpoB
Isoniazid	inhA, katg codon 315
Ethionamid	inhA, ethA, ethR
Ethambutol	embB codon 306
Pyrazinamide	pncA
Streptomycin	rpsL (43, 88), rrs region 530, gidB
Kanamycin, Amikacin	rrs region 1401 and 1490, eis
Capreomycin	rrs region 1401 and 1490, tlyA
Fluoroquinolones	gyrA (codons 88 to 94), gyrB
PAS	thyA?folC? folP, dfrA
Cycloserine	??
Linezolid	rrl
Bedaquiline	atpE, Rv0678



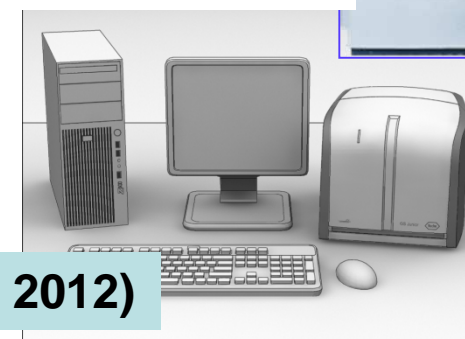
**Data bases**

**Web sites,**

<https://tbdreamdb.ki.se/>

[www.broadinstitute.org](http://www.broadinstitute.org)

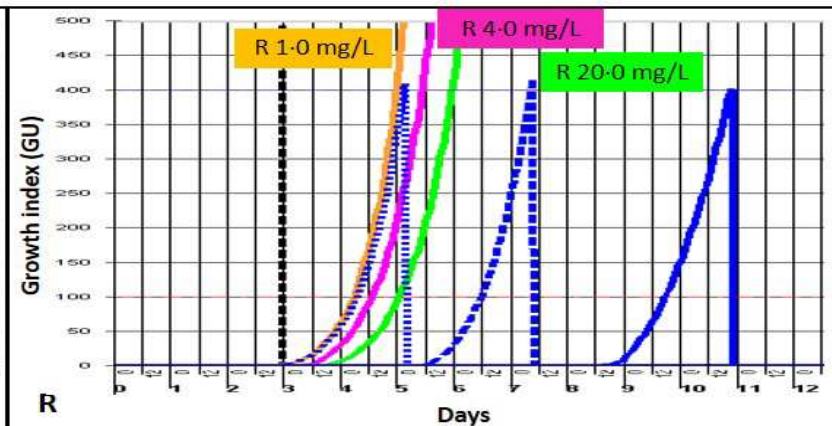
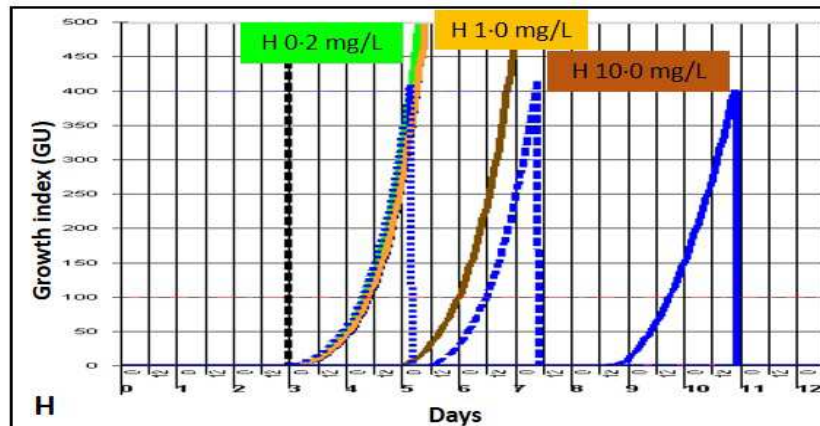
MUBII-TB-DB...



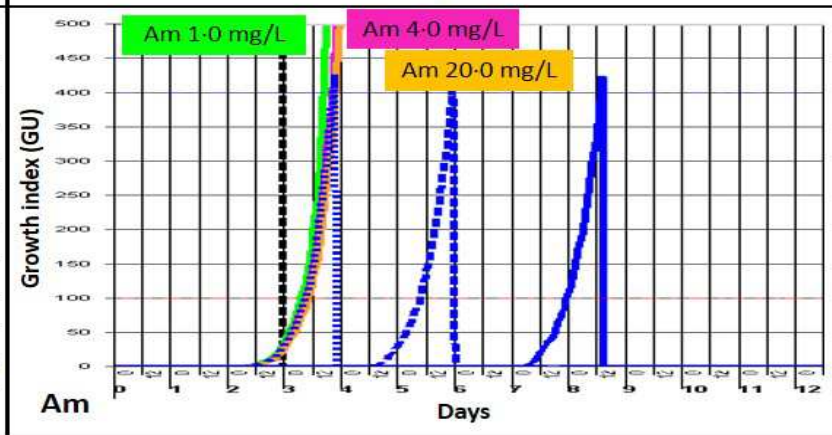
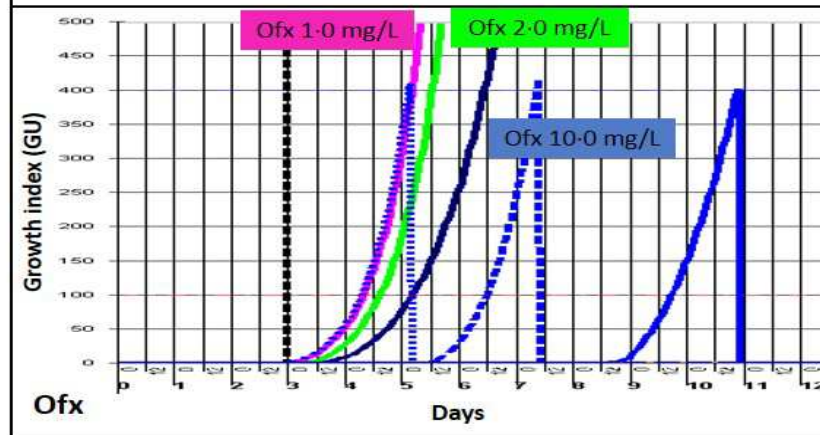
Daum LT 2012, Niemann 2009, Comas I 2011, Liu cui Hua 2012)

# Individualized treatment based on super-phenotypic drug susceptibility testing with the MGIT-TBeXiST protocol

MDR  
detection



XDR  
detection



Bottger et al. 2013, Cambau et al. JAC 2015; Gulglielmetti et al. submitted

# Towards having new anti-TB drugs

- Research of new compounds ongoing
- Mechanisms of action and resistance
- EUCAST-ESGMYC working group in progress for assessing in vitro activity
  - MIC reference methods and labs
  - Test on non MDR strains for biosafety
- EMA guidelines for redesign clinical studies (endpoints for TB drug efficacy)

# Genotypes of MDR-TB isolates in France (2006-2014)

Lignée	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
<b>Groupe PGG1</b>										
EAI	2	1	0	0	0	0		2	3	8
CAS-DELHI	0	0	0	1	0	3	4	1	1	10
Beijing	11	9	15	17	19	23	38	41	52	225
West African 2	1	1	0	0	0	0	1	1		4
West African 1	0	0	0	0	0	1				1
Canettii	1	0	0	0	0	0				1
<b>Groupe PGG2</b>										
Haarlem et X	5	0	2	2	2	3	2	10	9	35
LAM	5	7	3	3	4	8	16	10	19	75
URAL	4	0	2	1	0	1	2	3	4	17
TUR (Ural)	1	0	0	1	0	0		1		3
S	2	2	3	1	3	3	3	2	1	20
Cameroon	2	2	3	1	3	1	3			15
Ghana (T1)	4	1	5	6	2	3	1	1	3	26
T1-T2-T3 et « nt »	19	14	18	19	12	19	21	11	18	151
<b>Divers</b>										
NEW-1	0	0	0	0	0	1				1
Bovis	0	0	0	0	0	1				1
<b>Total</b>	<b>57</b>	<b>37</b>	<b>51</b>	<b>52</b>	<b>45</b>	<b>67</b>	<b>91</b>	<b>83</b>	<b>110</b>	<b>593</b>

Rapport CNR 2015, <http://cnrmyctb.free.fr>

# Genotypes of MDR-TB isolates in France (2006-2014, Report NRC 2015)

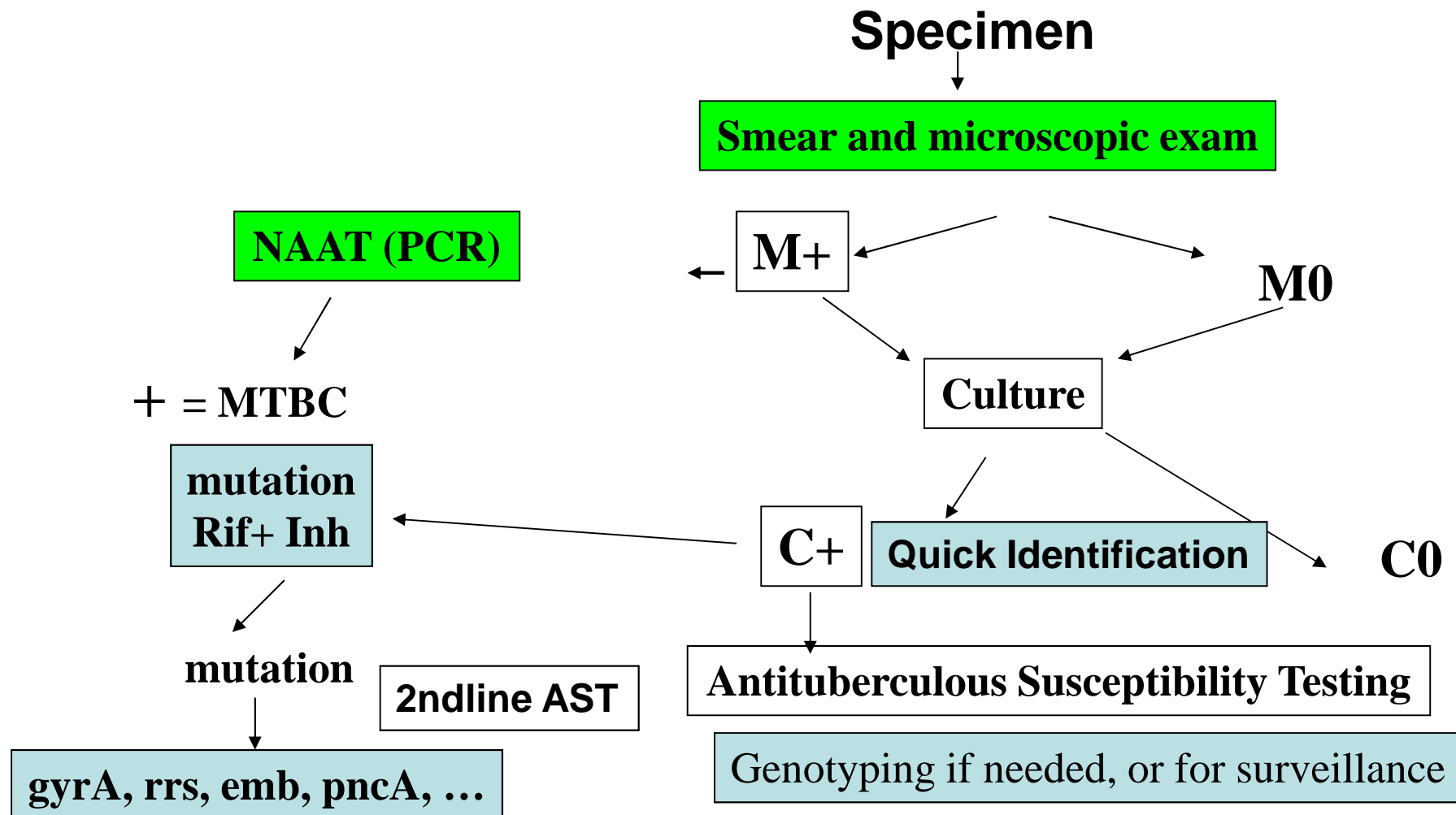
Lignée	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
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- **Increase in Beijing lineage (19 to 47%)**
- **Half of isolates are distributed in small clusters showing family or local transmission**
- **In 2014 among 110 MDR-TB isolates, 51 (46%) were from patients coming from Russia and central Asia**
- **Genotyping with MIRU24 (ECDC protocol):**
  - **33 Beijing lineage from Georgia (25), Russia (4), Ukraina (2), Tchechenia (1), Armenia (1)**
  - **3 LAM lineage from Georgia (2), Ukraina (1)**
  - **2 URAL lineage from Armenia**

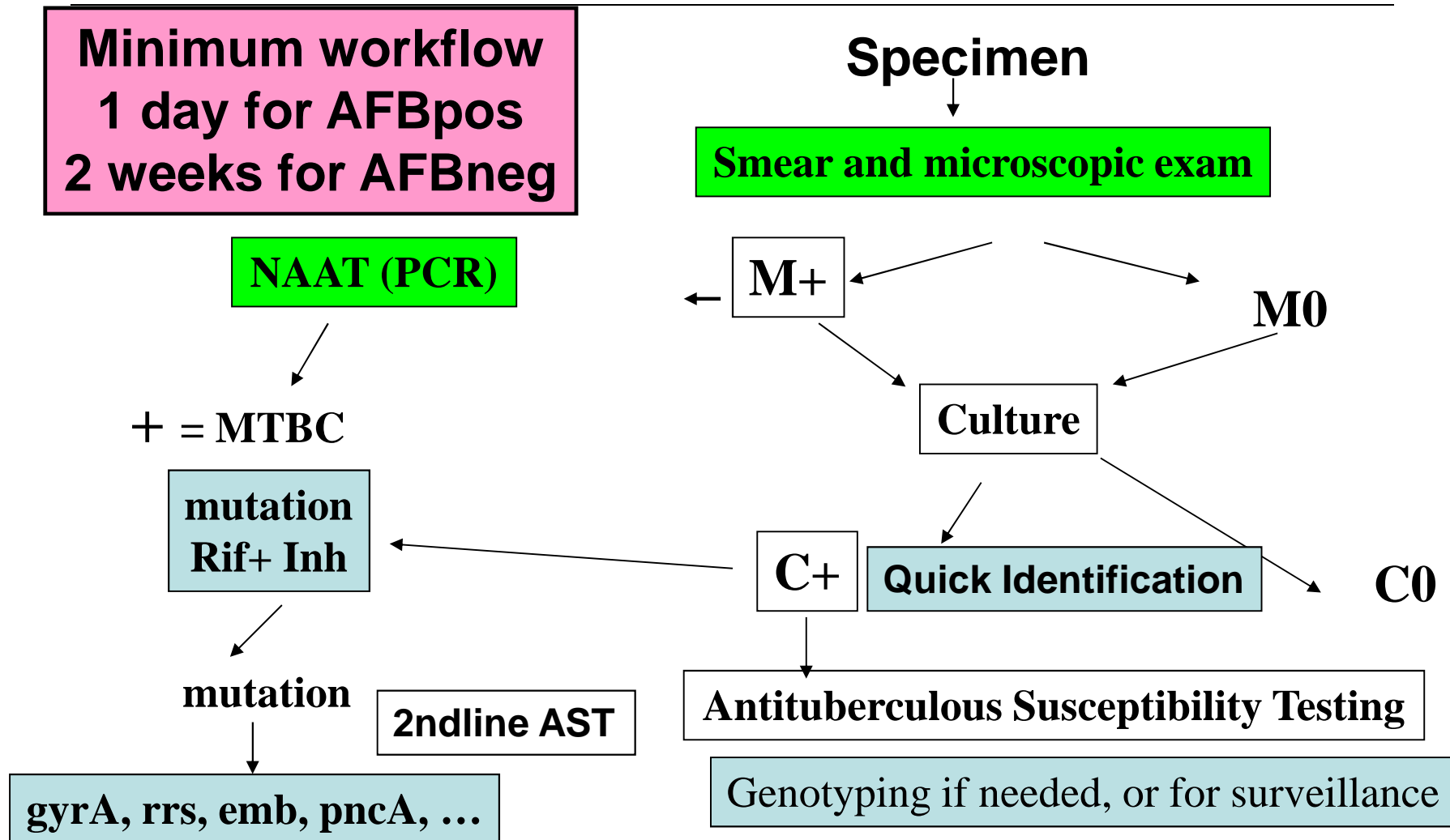
Total	57	37	51	52	45	67	91	83	110	593
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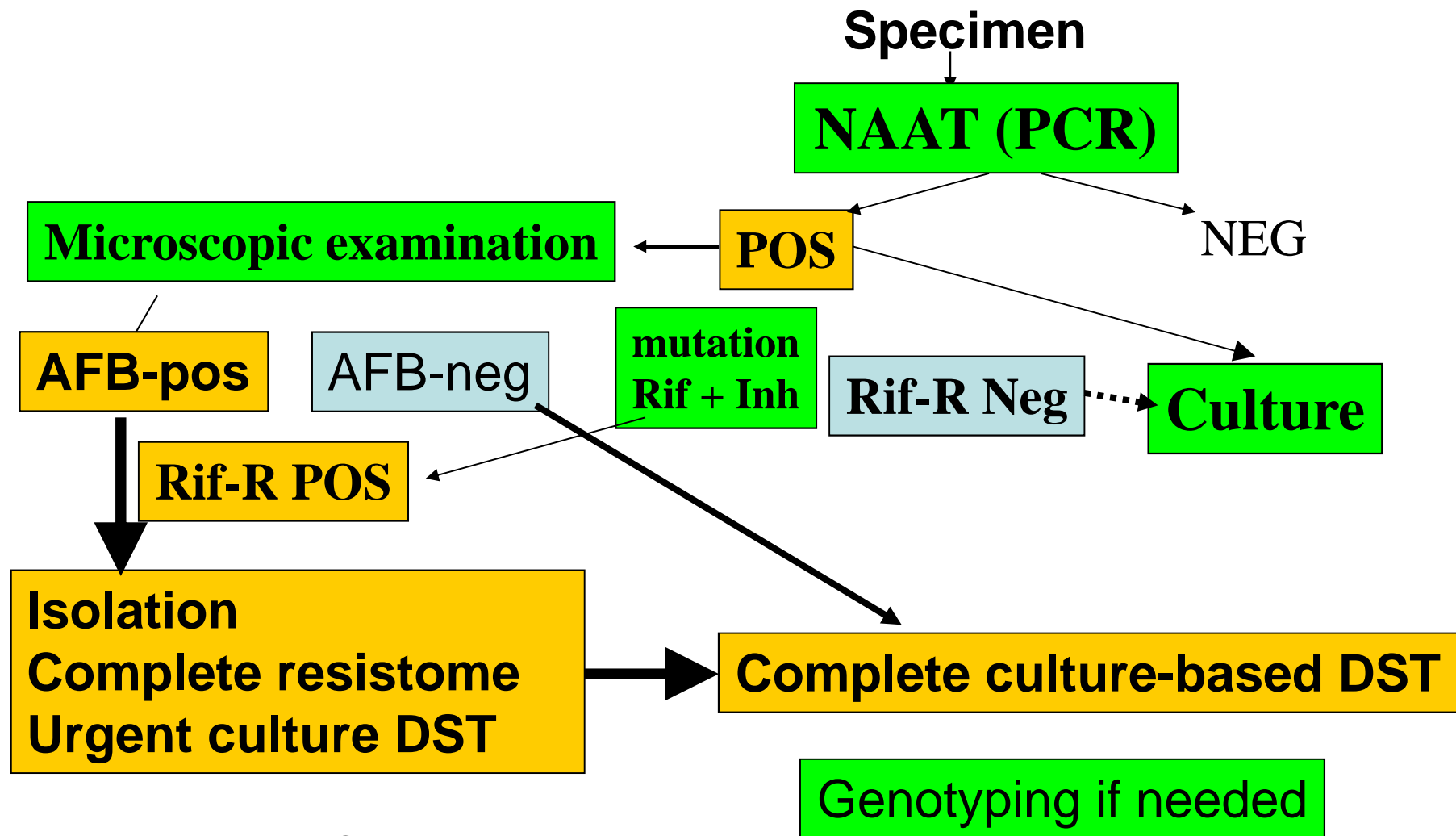
# Strategy « smear-first » for diagnosis of MDR-tuberculosis (ECDC – ERLTB net)



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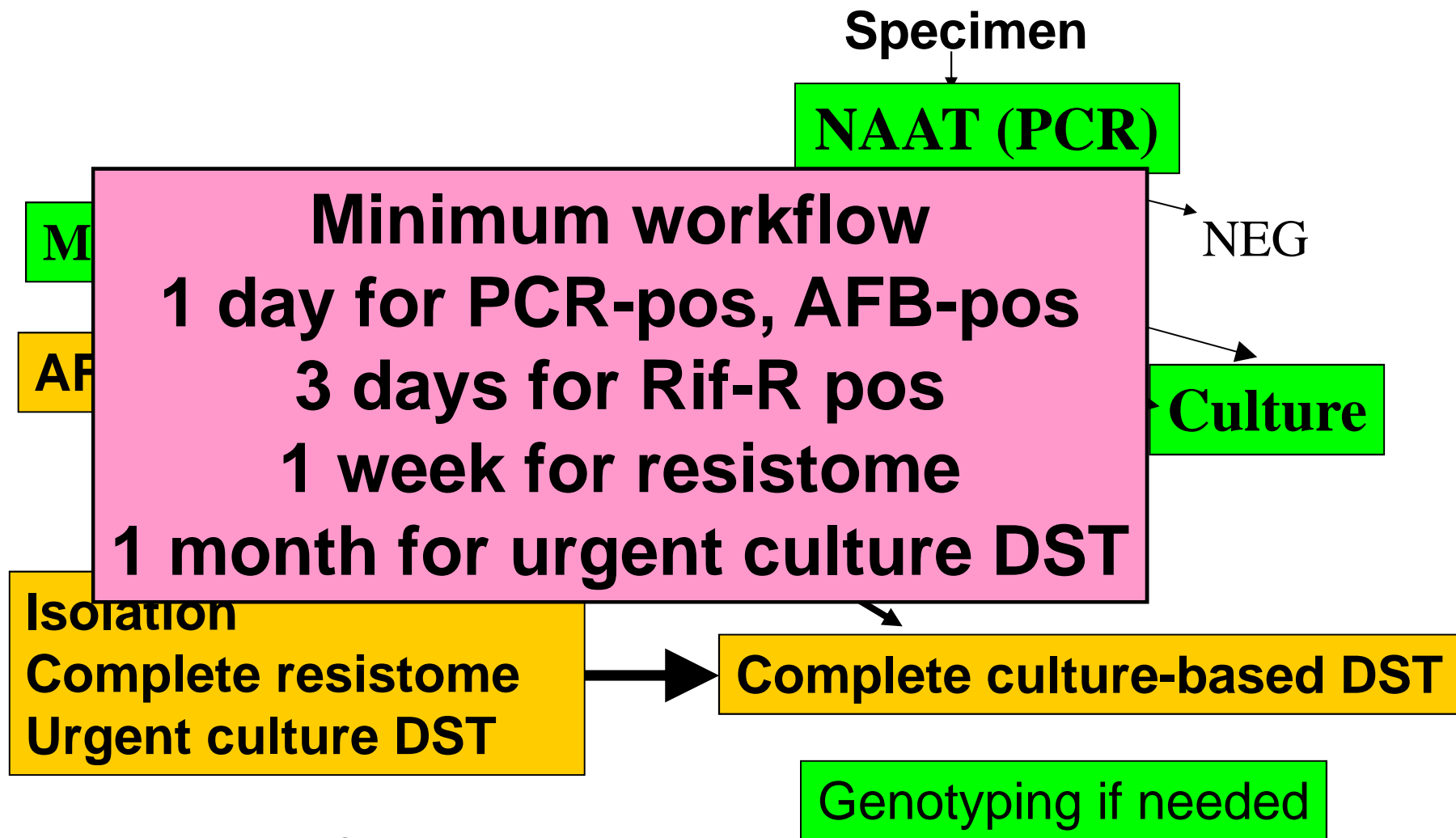


# Strategy « PCR-first » for bacteriological diagnosis of MDR-tuberculosis (WHO- FIND)



Adapted from WHO publications

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Adapted from WHO publications

# Conclusions

- Cooperation between clinicians, patients, and clinical laboratories
- Strategies according to the epidemiology and the health organization of the city/region/country
- Rapid screening of MDR-TB patients (PCR) balanced by long-term investment on molecular and culture-based drug susceptibility testing (DST) to gain effective individualized treatments.
- Mandatory research: diagnosis, drugs human and social sciences, co-infections and co-morbidities



# Serology for tuberculosis

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- 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
<b>Pulmonary TB</b>				
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 <sup>a</sup>	12	1,231 (1,512)	53 (42–64)	98 (94–99)
<b>Extrapulmonary TB</b>				
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.

<sup>a</sup>Serological 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



# Speciation within the *M. tuberculosis* complex

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

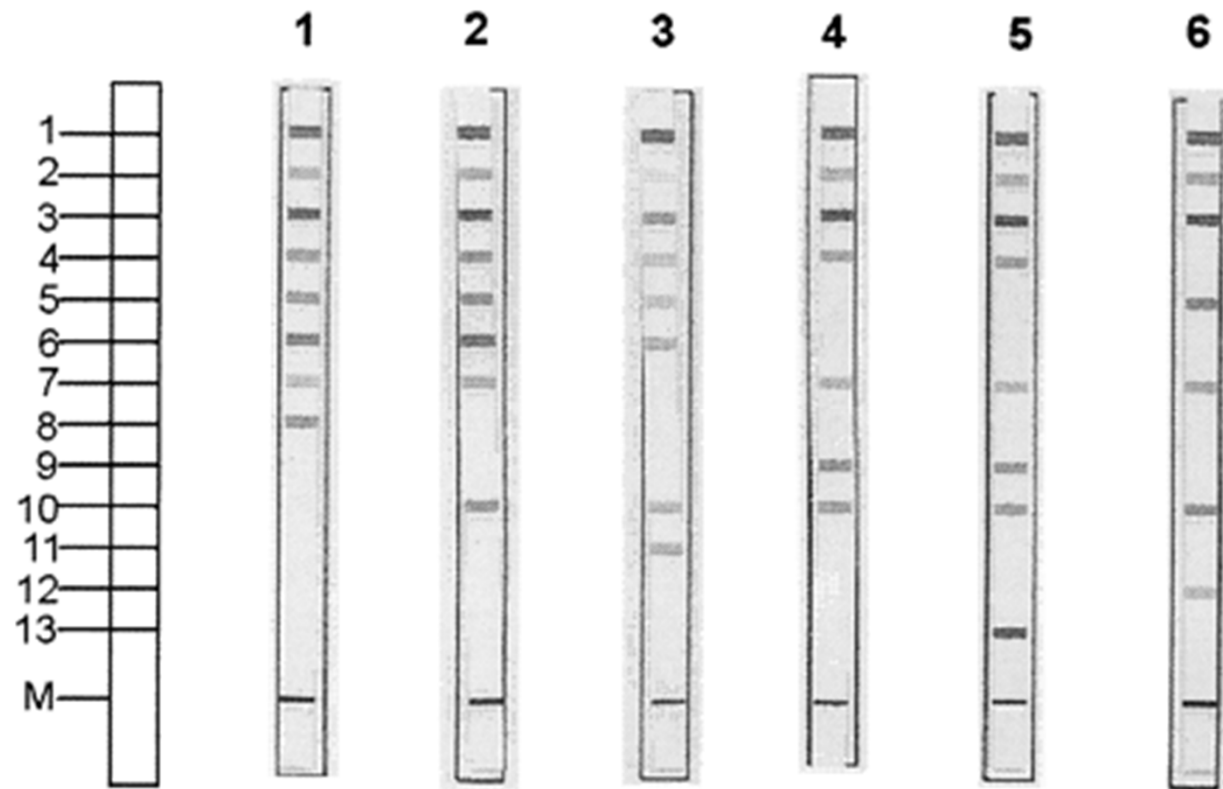
2. *M. africanum* I

3. *M. microti*

4. *M. bovis*

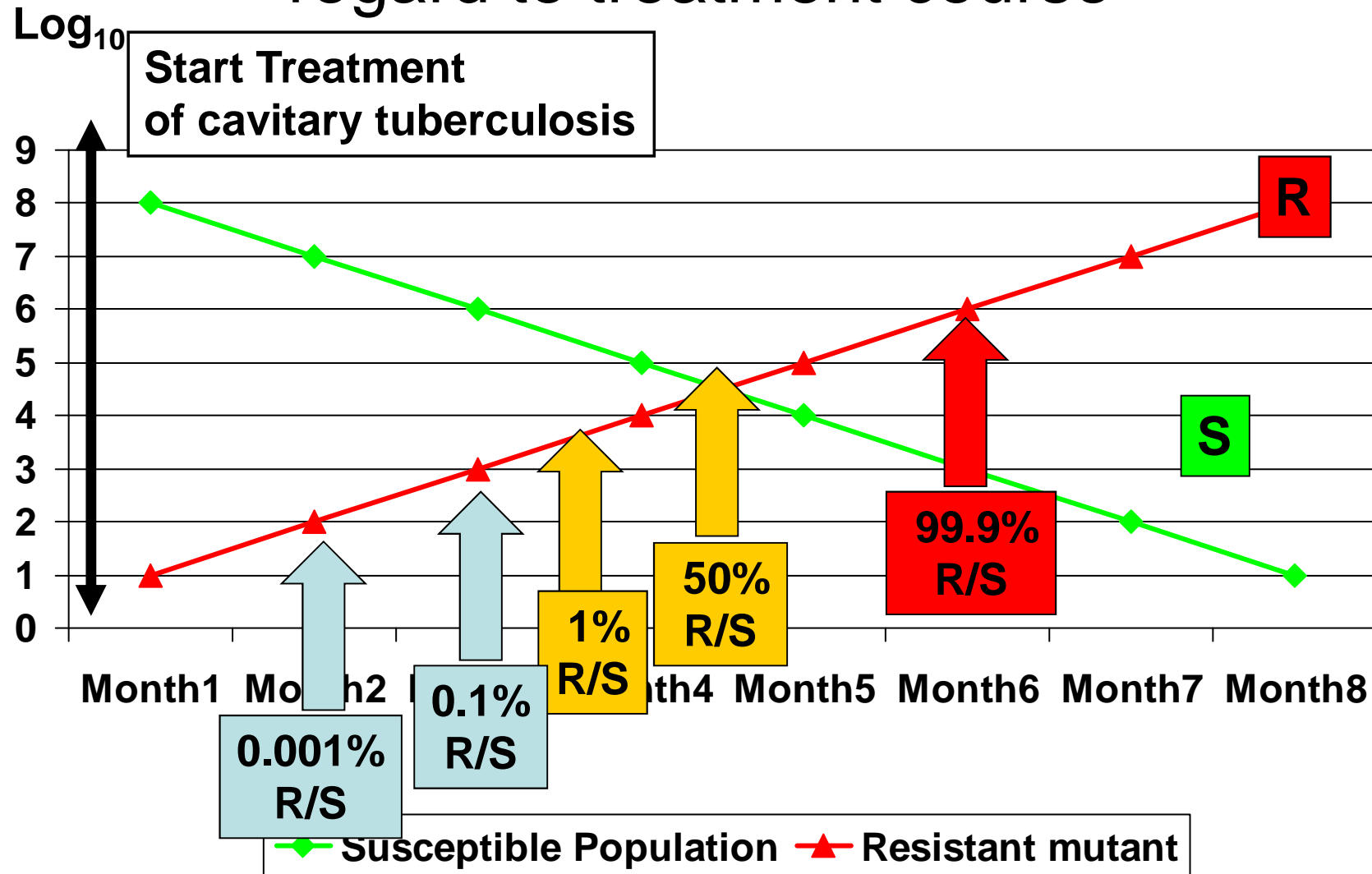
5. *M. bovis* BCG

6. *M. bovis* caprae



**genoTypeMTBC® (Hain Lifescience)**

# Various proportions of resistant mutant with regard to treatment course



Emmanuelle CAMBAU

