New diagnostic methods for mycobacteria : the pros and cons

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CiMi, INSERM, Sorbonne Université

Conflicts of interest

- Janssen
- Otsuka
- Becton Dickinson

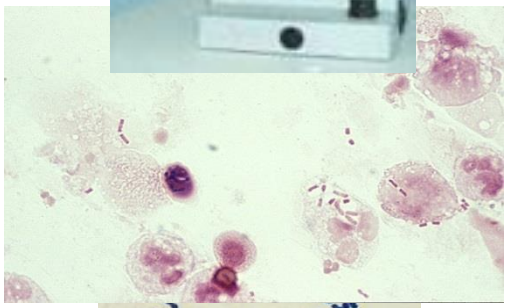
Diagnosis of tuberculous disease

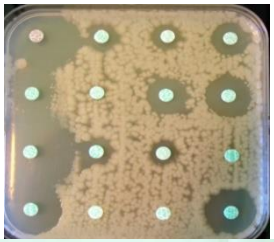
Usual bacteriological diagnosis

D0
microscopy

D1
Culture/identification

D2
DST



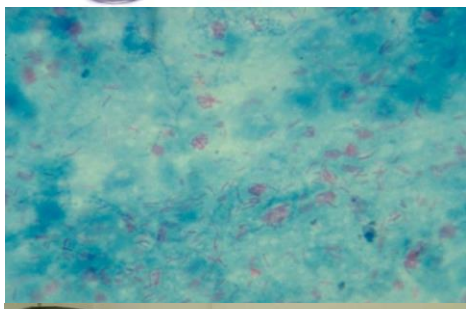


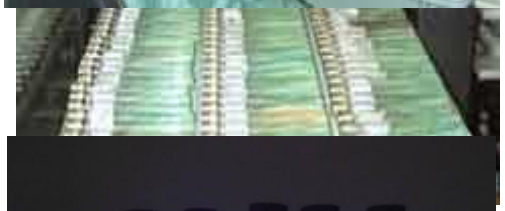
Tuberculosis bacteriological diagnosis

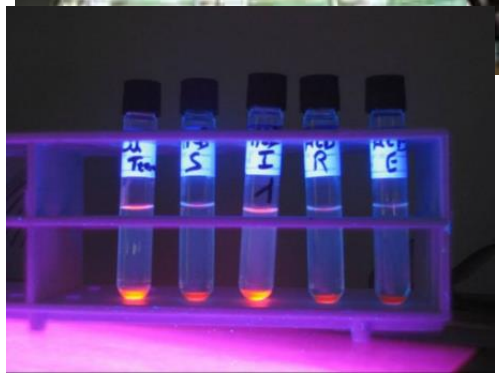
D0
microscopy

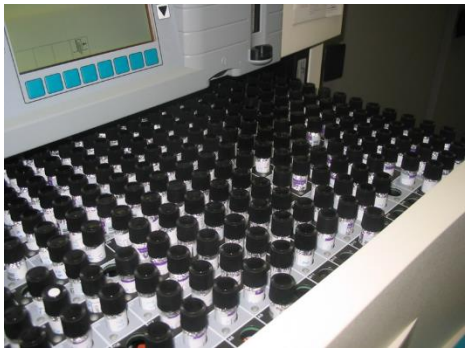
D3-M3
culture / identification

M1-2
DST

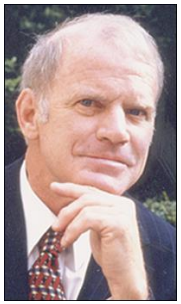




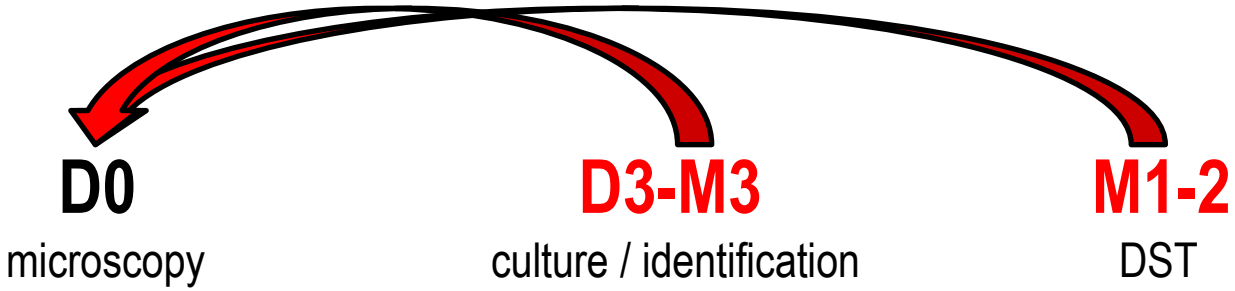




Polymerase chain reaction



- Karry Mullis 1983
- Amplifies the number of copies of nucleic acids in order to allow thier detection
- Theorically allows detection of 1 DNA molecule
- Great hope for a fast diagnosis of tuberculosis from samples



PCR performances for the diagnosis of tuberculosis

Tuberculosis	Sensitivity	Specificity	Prevalence	PPV	NPV
Smear +	98%	98%	85%	98%	90%
Smear -	72%	96%	5%[a]	?	?
			2%[b]	?	?
Extra-respiratory (smear-)	30%	98%	0.5%	?	?

a : respiratory and ID ward, b : other wards

Sarmiento, JCM 2003

PCR performances for the diagnosis of smear negative tuberculosis

Se = 72% Sp = 96%	Culture +	Culture -
Prevalence = 5%	5	95
PCR +	**3.6** (5x0.72)	**3.8** (95x0.04)
PCR -	**1.4** (5x0.28)	**91.2** (95x0.96)

What can be done?

PPV = 3.6/(3.6+3.8)
= 49%

NPV = 91.2/(91.2+1.4)
= 98%

PCR : evolution of performances

Reference	Methodology	Technology	Sensitivity among smear negative pulmonary specimens
Sarmiento, JCM 2003	Meta-analysis	many	72%

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

« At sites peforming alternatives nucleic acid-amplification testing, the sensitivity of the MTB/RIF test performed diectly on sputum was higher than that of Amplicor and similar to that of ProbeTec »

PCR : evolution of performances

Reference	Methodology	Technology	Sensitivity among smear negative pulmonary specimens
Sarmiento, JCM 2003	Meta-analysis	many	72%
Boehme, NEJM 2010	Prospective study	Xpert MTB/RIF	72%
Steingart, Cochrane 2014	Meta-analysis	Xpert MTB/RIF	67%

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study

Susan E Dorman, Samuel G Schumacher*, David Alland, Pamela Nabeta, Derek T Armstrong, Bonnie King, Sandra L Hall, Soumitesh Chakravorty, Daniela M Cirillo, Nestani Tukvadze, Nino Bablishvili, Wendy Stevens, Lesley Scott, Camilla Rodrigues, Mubin I Kazi, Moses Joloba, Lydia Nakiyingi, Mark P Nicol, Yonas Ghebrekristos, Irene Anyango, Wilfred Murithi, Reynaldo Dietze, Renata Lyrio Peres, Alena Skrahina, Vera Auchynka, Kamal Kishore Chopra, Mahmud Hanif, Xin Liu, Xing Yuan, Catharina C Boehme, Jerrold J Ellner, Claudia M Denkinger, on behalf of the study team†*

Lancet Infect Dis 2017

« For tuberculosis case detection, sensitivity of Xpert Ultra was superior to that of Xpert in patients with paucibacillary disease »

PCR : evolution of performances

Reference	Methodology	Technology	Sensitivity among smear negative pulmonary specimens
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Boehme, NEJM 2010	Prospective study	Xpert MTB/RIF	72%
Steingart, Cochrane 2014	Meta-analysis	Xpert MTB/RIF	67%
Dorman, LID 2018	Prospective study	Xpert Ultra	63%
		Xpert MTB/RIF	46%

Beware of announcement!

Xpert MTB/RIF : real life

- Lee, AJRCCM 2019 : diagnostic performances of Xpert MTB/RIF
- Korea (TB incidence $77/10^5$)
- Consecutive sputum samples collected from 2,952 suspected pulmonary tuberculosis patients over a 3-year period
  - "Xpert provides faster, more stable, and superior results compared with smear microscopy, in addition to its strong correlation with smear grade. Xpert might replace smear microscopy as the first-line diagnostic test for pulmonary tuberculosis in routine clinical practice in an intermediate-burden setting"

Assay	Heterogeneity Factor	Diagnostic Performance (95% CI)			
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Xpert MTB/RIF	Smear-positive	96.1 (90.3–98.9)	92.2 (84.6–96.8)	93.3 (87.3–96.6)	95.4 (88.8–98.2)
	Smear-negative	60.2 (52.3–67.9)	97.7 (97.1–98.3)	62.2 (55.4–68.5)	97.5 (97.0–98.0)
		$P < 0.0001$	$P = 0.0009$	$P < 0.0001$	n.s.

PPV limited for tuberculosis

PPV : Ultra?

- Dorman, LID 2018 : Ultra vs MTB/RIF

	Sensitivity		Specificity		
	All culture-positive (95% CI; n/N)	Smear-negative, culture-positive (95% CI; n/N)	All culture-negative (95% CI; n/N)	No history of tuberculosis (95% CI; n/N)	Any history of tuberculosis (95% CI; n/N)
Xpert	83% (79–86; 383/462)	46% (37–55; 63/137)	98% (97–99; 960/977)	98% (97–99; 715/727)	98% (95–99; 244/249)
Xpert Ultra	88% (85–91; 408/462)	63% (54–71; 86/137)	96% (94–97; 934/977)	96% (95–98; 701/727)	93% (89–96; 232/249)

Ultra = lower specificity ⟶ lower PPV

Limits of molecular methods : Epidemiology and pre-test probability

Lesotho

Incidence : 724 / 100 000 → **1%**

	TB	No TB
	1	99
Xpert +	0.89 (1 x 89%)	0.99 (99 x 1%)
Xpert -	0.11 (1 x 11%)	98.01 (99 x 99%)

PPV = **47%**

NPV = 99%

Genotypic diagnosis not efficient if used without clinical suspicion

Limits of molecular methods : Epidemiology and pre-test probability

France, high clinical suspicion

Incidence : 7.7 / 100 000 → **0.01%**

HIV+ and homeless (**RR 300**), cough> 2 weeks (**RR 4**)

	TB	No TB	
	0.01*300*4 = 12	88	
Xpert +	10.68 (12 x 89%)	0.88 (88 x 1%)	**PPV** = **92.4%**
Xpert -	1.32 (12 x 11%)	87.12 (88 x 99%)	**NPV** = 98.5%

Genotypic diagnosis efficient if used following a clinical algorithm

Diagnosis of tuberculosis: Conclusion

- Genotypic diagnosis to be integrated in a global strategy (no angling fishing)

Diagnosis of drug resistance

A long time ago in a galaxy far, far away....

Revue de Tuberculose et de Pneumologie. T. 27, 1963, n° 2-3 (pp. 217-272).

MESURE DE LA SENSIBILITÉ DU BACILLE TUBERCULEUX
AUX DROGUES ANTIBACILLAIRES
PAR LA MÉTHODE DES PROPORTIONS.

MÉTHODOLOGIE, CRITÈRES DE RÉSISTANCE, RÉSULTATS, INTERPRÉTATION

par

G. CANETTI, N. RIST et J. GROSSET
(Institut Pasteur, Paris).

Reference for phenotypic diagnosis of resistance
Adapted to liquid media in the 80s

From phenotype to genotype

Phenotypic methods

LIMIT : *M. tuberculosis* is a slow grower

$\Rightarrow$ Room for genotypic tests

Study of genes coding proteins implied in drug resistance

Genotypic diagnosis of drug resistance: the first study

Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*

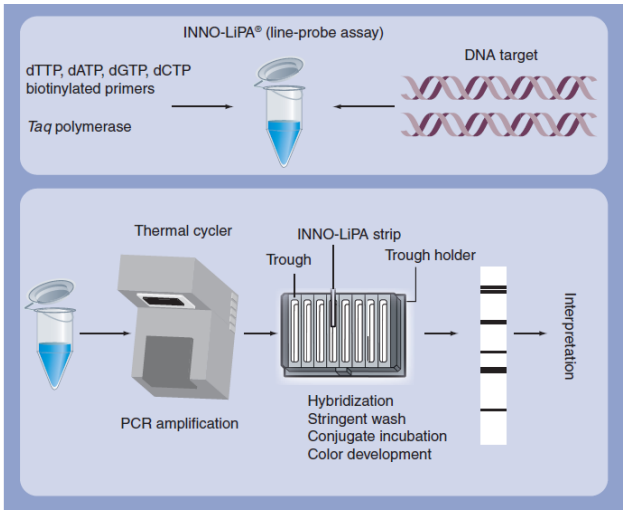
AMALIO TELENTI PAUL IMBODEN FRANCINE MARCHESI
DOUGLAS LOWRIE STEWART COLE M. JOSEPH COLSTON
LUKAS MATTER KURT SCHOPFER THOMAS BODMER

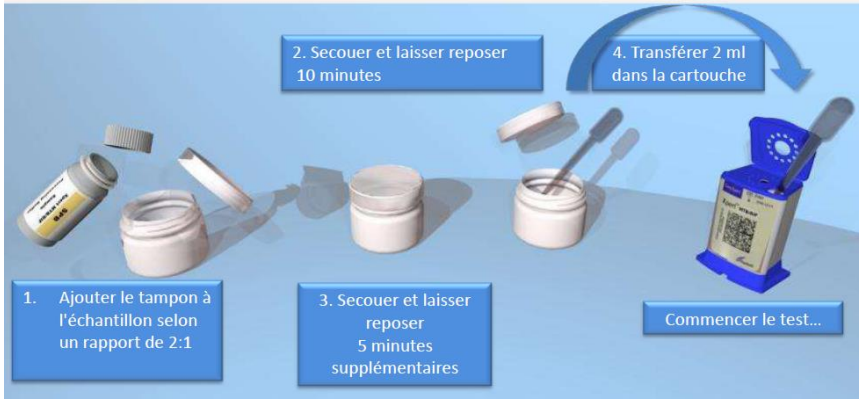
Lancet 1993; **341**: 647–50.

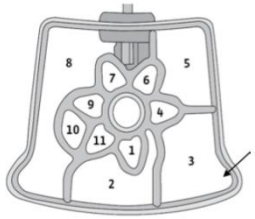
- 122 clinical strains of *M. tuberculosis*
- *rpoB* Amplification/sequencing
- No mutation in 56 susceptible strains (sequence identical to H37Rv)
- 64/66 resistant strains with *rpoB* mutations

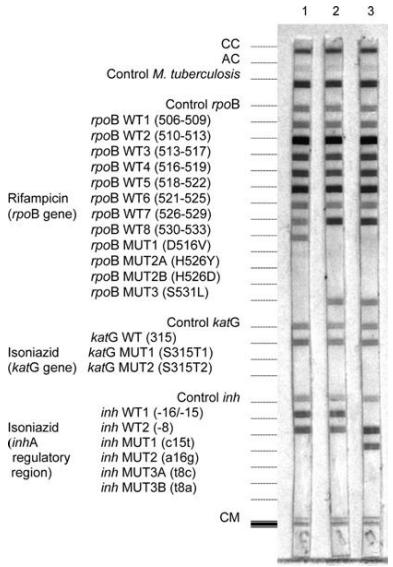
First demonstration of the possibility of a genotypic diagnosis of drug resistance
Excellent prediction of resistance (64/64 mutated are resistant = 100%)
Good prediction of susceptibility (56/58 non mutated are susceptible = 97%)

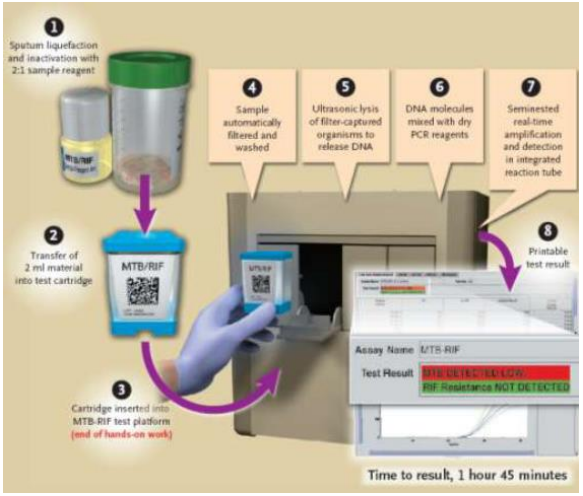
MTBDR, Xpert MTB/RIF











Genotypic tests accelerate the diagnosis of resistance

Drug	Gene	test	Sensitivity	Specificity	Performances
Rifampin	*rpoB*	MTBDR*plus*	98%	99%	Excellent
		Xpert MTB/RIF	94%	98%	
Isoniazid	*inhA, katG*	MTBDR*plus*	84%	99%	Good
Fluoroquinolones	*gyrA, gyrB*	MTBDR*sl*	83%	97%	Good
Amikacine	*rrs, eis*		87%	99%	Good
Kanamycin			67%	98%	Poor
Capreomycin			79%	95%	Good
Ethambutol	*embB*		68%	80%	Poor

Theron, 2014; Steingart 2013 ; Feng 2013 ; Ling 2008

Performances

-Excellent for rifampin→recommandation by HCSP 2015 for each new TB case

- Good for isoniazid, fluoroquinolones, amikacin, capreomycin

- Poor for kanamycin and ethambutol (improved in MTBDR*sl* V2)

Whole genome sequencing

- Makhado, Lancet ID, 2018
- *rpoB* mutation Ile491Phe not screened by commercial tests
- Restrospective analysis of 1823 isoniazid resistant and rifampin susceptible strains
- 277 strains randomly selected for study
- Deeplex-MycTB deep sequencing
  - 37 with rpoB Ile491Phe mutation = MDR

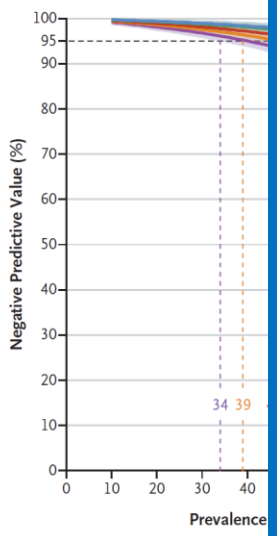
WGS detects low-level rifampin resistance better than current genotypic tests and than phenotypic DST
Is WGS able to predict susceptibility?

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 OCTOBER 11, 2018 VOL. 379 NO. 15

Prediction of Suscepti
by
The CRyPTIC C

10 209 *M. tu*

Detection (%)
R
S



Beware of announcement!

10 209 genomes

↓

7516 complete DST

↓

5865 interpretable genomes

↓

5250 concordant with DST

=**70%** of strains with DST

= **51%** of genomes

Proof of concept but not of feasibility in real life

eptibility
revalence

Genotype/Phenotype correlation: fluoroquinolones

- Bernard, AAC 2015 : Prospective study of *gyrA* and *gyrB* mutations associated with fluoroquinolone resistance in *M. tuberculosis* strains
  - 605 strains received at the NRC between 2007 and 2012
    - Mutations *gyrA* : 78% associated with resistance
    - Mutations *gyrB* : 36% associated with resistance

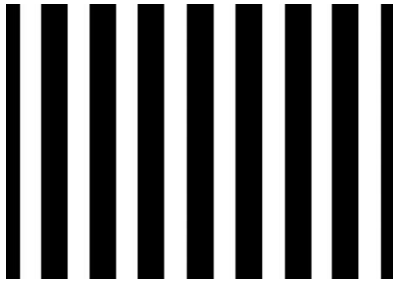
- Aubry PLOS One 2014, Pantel, JAC 2016 :
  - GyrA A90G substitution confers quinolone susceptibility

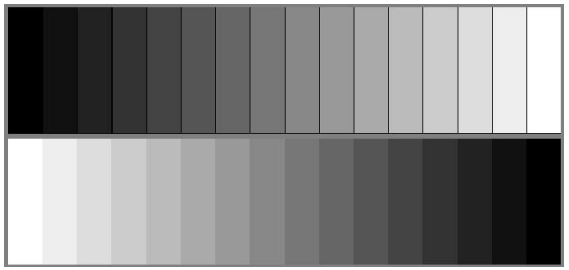
- Maitre, JAC 2016 :
  - Some GyrB mutations phenotypically classified « S » reduce the *in vivo* activity of fluoroquinolones

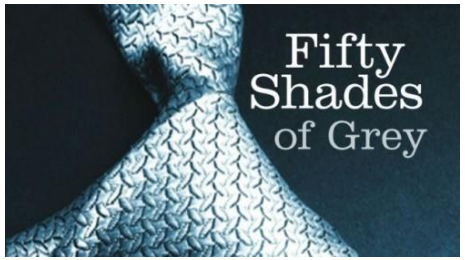
Genotypic diagnosis sheds light on the variability of drug resistance levels

Diagnosis of resistances: Conclusion

- Phenotypic tests remain the gold standard
- Genotype/Phenotype correlation
  - Work in progress
  - Including impact on treatment success
  - Go from black and white to shades of grey







General conclusion

- Genotypic diagnosis accelerates diagnosis of tuberculosis and of drug resistance
- Know the limits for a good use in routine
- Tomorrow, WGS in routine?

Oana Dumitrescu

- Clinical use of whole genome sequencing for Mycobacterium tuberculosis in the workflow of TB diagnosis and control

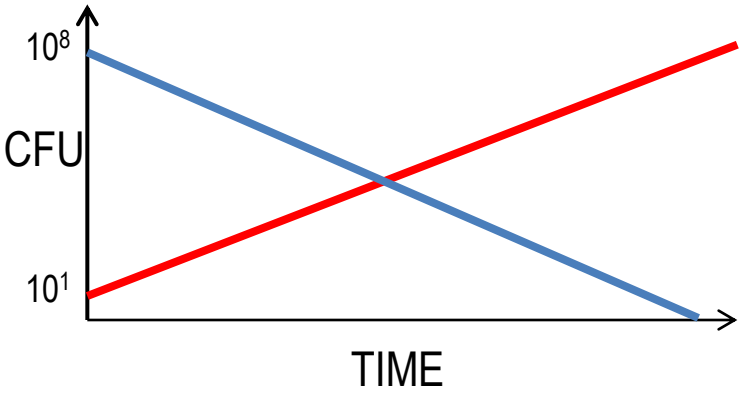
Isabelle Bonnet

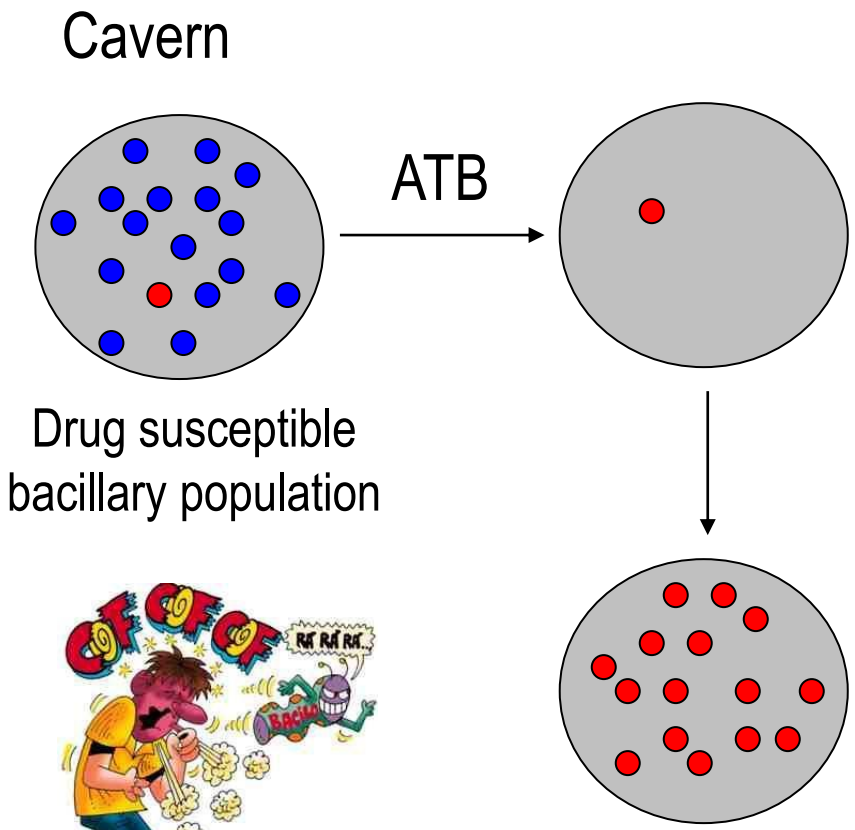
- First evaluation in routine use of the combination of GeneLeadVIII to extract and detect Mycobacterium tuberculosis (Mtb) DNA and Deeplex-MycTB to predict drug resistance and TB transmission in less than 7 days from clinical samples

Mechanism of selection of drug resistant mutants

- Susceptible bacilli
- Drug resistant bacilli

Antibiotic	Concentration	Mutant proportion
pyrazinamide	100mg/L	10-5
isoniazide	0.2mg/L	10-6
streptomycin	2mg/L	10-6
rifampin	1mg/L	10-8
bedaquiline	0.5mg/L	10-8
linezolide	8mg/L	10-9



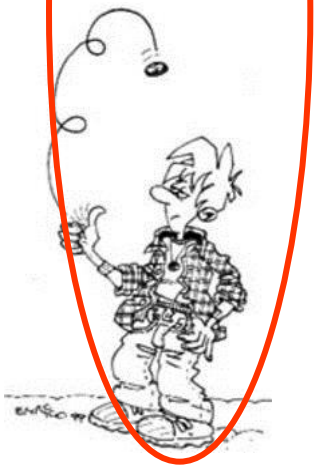


Is an *rpoB* mutation always predictive of drug resistance?

Sensitivity= 100% and specificity = 98%, what PPV do we get?

		France	
		Previously treated	Not previously treated
Prevalence of resistance	30%	9%	2%
N R strains for 1000 patients	300	90	20
N false positive for 1000 tests (Sp=98%)	20	20	20
PPV	94%	82%	50%

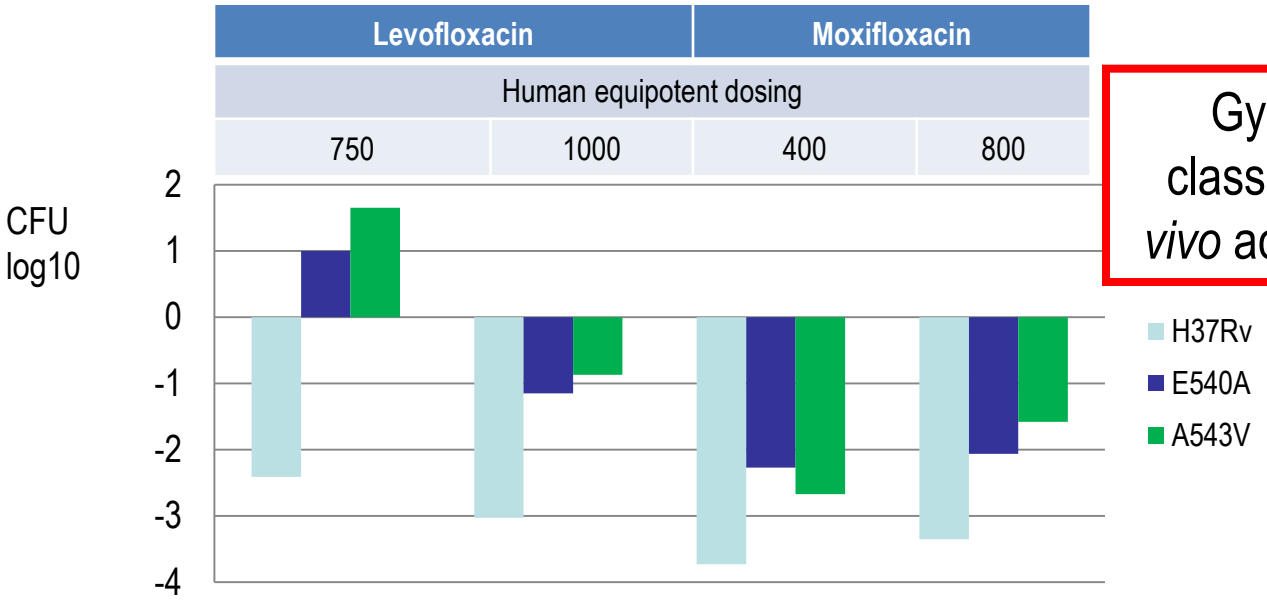
Good test for MDR screening
PPV depends of pre-test probability
In case of low pre-test porbability → repeat test



Fluoroquinolone low-level resistance

Genotype		Phenotype : MIC (µg/ml)	
GyrA	GyrB	levofloxacin	moxifloxacin
WT	WT	≤ 0.25	≤ 0.25
WT	E540A	0.5	0.5
WT	A543V	1	0.5

« Oficially» susceptible
Critical Concentrations
- Levofloxacin = 1 mg/L
- Moxifloxacin = 0.5 mg/L



GyrB mutants with MICs classified « S » reduce *the in vivo* activity of fluoroquinolones

Maitre, JAC 2016

An example of the superiority of genotypic diagnosis over phenotypic diagnosis

Whole genome sequencing

- Papaventsis, CMI 2017 : literature review

Table 1
Drugs tested in the reports [4–7,12,14,22–35], genes associated with drug resistance and whole genome sequencing performance characteristics[a]

Drug	No of studies	No of strains	Genes and other relevant *Mycobacterium tuberculosis* genome regions	Sensitivity, % (range)		Specificity, % (range)	
				Low	High	Low	High
Rifampicin	19	6286	*rpoB, rpoA, rpoC*	89.2	100.0	66.7	100.0
Isoniazid	19	5800	*katG, inhA, oxyR-ahpC, fpbC, Rv1592C, Rv1772, Rv2242, fabD, fabG1, kasA, accD, oxyR, ndh, fadE24, nat, kasA, mabA, p_inhA, accD6, efpA*	90.0	100.0	83.3	100.0
Ethambutol	17	6059	*embA, embB, embC, embR, iniA, iniB, iniC, Rv3124, manB, PPE49, rmlD, manB*	71.4	100.0	15.4	95.8
Pyrazinamide	13	6130	*pncA, p_pncA, rpsA, panD*	43.2	100.0	66.7	100.0
Streptomycin	16	3953	*rpsL, rrs, gidB*	57.1	100.0	40.0	100.0
Amikacin[b]	8	1471	*rrs, eis, gidB, tlyA*	80.0	100.0	50.0	100.0
Capreomycin[b]	8	1553	*rrs, eis, gidB, tlyA*	60.7	100.0	13.7	100.0
Kanamycin[b]	7	1289	*rrs, eis, tlyA*	75.0	100.0	0	100.0
Injectable drugs[d]	4	518	*rrs, eis, gidB, tlyA*	37.0	100.0	50.0	100.0
Ciprofloxacin	1	300	*gyrA, gyrB*	100.0	100.0	98.9	98.9
Ofloxacin[c]	6	1564	*gyrA, gyrB*	80.0	100.0	80.0	100.0
Moxifloxacin[c]	3	1318	*gyrA, gyrB*	60.0	90.9	68.7	100.0
Levofloxacin[c]	-	—	*gyrA, gyrB*	—	—	—	—
Gatifloxacin[c]	-	—	*gyrA, gyrB*	—	—	—	—
Fluoroquinolones[e]	9	504	*gyrA, gyrB*	89.2	100.0	100.0	100.0
Ethionamide	8	867	*ethA, ethR, p_inhA, inhA, fabG1*	16.7	100.0	50.0	100.0
Prothionamide	3	502	*p_ethA, ethA*	40.0	100.0	29.4	80.0
Rifabutin	1	2	*rpoC*	100.0	100.0	—	—
Para-aminosalicylic acid[f]	1	11	*thyA, folC, ribB*	75.0	75.0	100.0	100.0
Trimethoprim/sulfamethoxazole	1	2	*dfrA*	—	—	100.0	100.0
Minocycline	1	2	*whiB7*	100.0	100.0	100.0	100.0
Linezolid	2	5	*Rrl, rplC*	—	—	100.0	100.0
Bedaquiline	—	—	*Rv0678*				
Clofazimine	—	—	*Rv0678*	—	—	—	—

Heterogeneous performances
Correlation with treatment issue?

PCR : evolution of performances

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Sarmiento, JCM 2003	Meta-analysis	many	72%
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Dorman, LID 2018	Prospective study	Xpert Ultra	63%
		Xpert MTB/RIF	46%
Chakravorty, Mbio 2017	Prospective and retrospective study	Xpert® MTB/RIF Ultra	79%
Opota, JCM 2019	Prospective study	Xpert® MTB/RIF Ultra	92%

Beware of announcement effects!