Tuberculosis diagnostics : Ancient and Modern (2,5hr)

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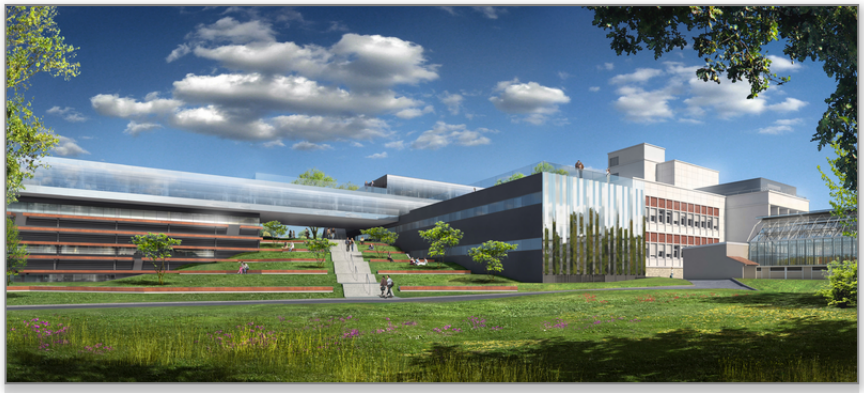
Presentation

The Institute of Integrative Biology of the Cell (I2BC) is a “Unité Mixte de Recherche” (mixed research unit ; UMR) supported by both Université Paris-Sud, the CNRS and the CEA.

The Institute regroup 80 teams of scientists, 15 technological facilities from 8 research units (CGM, IBBMC, IGM, ISV, LEGS, VMS, SB2SM, SBiGeM)

The Institute is in 3 research campus (Orsay Campus of the Université Paris Sud, Gif Sur Yvette Campus of CNRS, and Saclay Campus of CEA) with 14 buildings. All the I2BC activities will be join on the Gif Sur Yvette Campus in 2018.



I2BC Project

Our Missions are :

Global Health & Personalized Medicine

Anthropology, Microbiology, Genomics, Molecular Diagnostics, Bioinformatics, Public health, Economy

Tuberculosis

- Very ancient disease, paleopathology, abundant literacy
- Villemin Jean Antoine : demonstrates the transmissible nature of TB
- Koch Robert : discovers *M. tuberculosis* (and tuberculin)
- Disease at origin of development of Public health concept (sanatorium, mass vaccination)
- BCG = Bacille de Calmette-Guérin
- Waksman Selman : discovers Streptomycin in 1943
- Rifampicin (Rifamycin hemi-synthetic) 1967
- 1990: emergence of MDR TB- (« W » outbreak in NYC)
- 1994 TB= world-wide emergency of WHO
- today : 1,5 million death annually, co-infection VIH, 8 millions new cases/year

Introduction
A Mycobacteriology laboratory may have various levels of expertise

- **Type I**
  - >collect, perform **microscopy** examination, sample transmission, perform **GenXPert** when possible
- **Type II**
  - > equipment required to **isolate** and **culture MTBC**

  Identification, DST
- **Type III**
  - > all **NTM**, specific expertises
- **Type IV**
  - > SRLs, control of type III laboratories

Description of Biosafety cabinet
Requirements to isolate MTC : a P3 facility

- **Biosafety level 3 (**microbes which can cause serious and potentially lethal disease via the inhalation route)
- A medical surveillance
- All procedures involving infectious material must be done within a biological safety cabinet (BSL).
- Laboratory personnel must wear solid-front protective clothing (i.e. gowns that tie in the back). This cannot be worn outside of the laboratory and must be discarded or decontaminated after each use.
- A laboratory-specific biosafety manual must be drafted which details how the laboratory will operate in compliance with all safety requirements.
- The entrance to the laboratory must be separated from areas of the building with unrestricted traffic flow.
- Additionally, the laboratory must be behind **two sets of self-closing doors** (to reduce the risk of aerosols escaping).
- The construction of the laboratory is such that it can be easily cleaned.
- Carpets are not permitted, and any seams in the floors, walls, and ceilings are sealed to allow for easy cleaning and decontamination. Additionally, windows must be sealed
- a **ventilation system** installed which forces air to flow from the "clean" areas of the lab to the areas where infectious agents are handled.
- **Air** from the laboratory must be **filtered** before it can be recirculated.

What for use a BSL3 ?

- Biosafety level 3 is commonly used for research and diagnostic work involving various microbes which can be transmitted by aerosols and/or cause severe disease. These include:
- *Francisella tularensis*, *Mycobacterium tuberculosis*, *Chlamydia psittaci*, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, SARS coronavirus, *Coxiella burnetii*, Rift Valley fever virus, *Rickettsia rickettsii*, several species of *Brucella*, chikungunya, yellow fever virus, and West Nile virus.

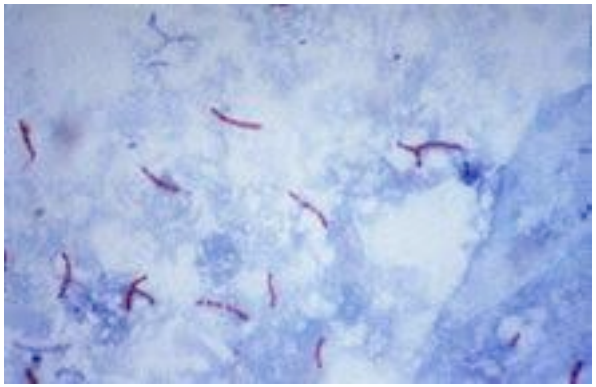
Sampling

- ***Expectoration/Sputa***
  - WHO changed its requirements:

    The revised definition of a new sputum smear-positive pulmonary TB case is based on the presence of at least one acid fast bacilli (AFB+) in at least one sputum sample in countries with a well functioning external quality assurance (EQA) system
- *Bronchio-alveolar washings*
- *Urine*
- *Tissues*
- *Blood, other (CSF, inflammatory liquids), stools*

Direct examination

- ZN or Kinyoun or Auramine colored smears is progressively replaced by GenExpert.



- More and more labs everywhere do not want to perform culture, which requires know-how, (BSL3), specialized staff.

- Molecular diagnostics and NGS could progressively kill mycobacterial culture capacities

TB Laboratory

1. Culture, Phenotypic Identification
2. Molecular identification

   16SrRNA, others
3. Liquid medium for drug susceptibility testing, phenotypic

   BACTEC MGIT 960, other methods
4. Sequencing for resistance detection, genotypic

   Rifampicin, Isoniazid, quinolones,
   Streptomycin, pyrazinamide, others
5. Other methods

   Diagnostics

   Molecular Epidemiology

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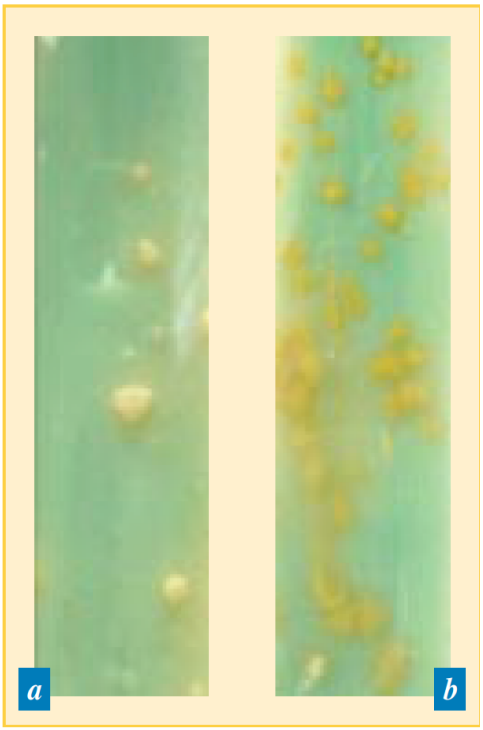
   - Molecular Epidemiology

Culture, Phenotypic Identification

Requires a pretreatment of the sputum (Kubicka, Petroff)

Medium :
Solid: **Löwenstein-Jensen, Middlebrook (7H10,7H11)**
Liquid : **Middlebrook (7H9)**

Time :
<7 days : rapid growers
>10 days: slow growers



TB **NTM**

Identification criteria, NTM versus MTC = time, pigment
M. bovis, M. africanum, M. tuberculosis
Growth in presence of :
Pyruvate, TCH, pyrazinamide, D-cycloserine

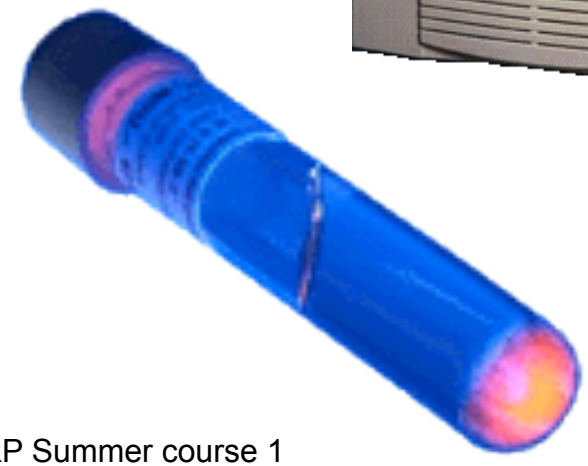
Biochemical properties
Niacin, Nitrate reductase

Before…Now

BACTEC™ System 460
Radiometric
^{14}C

BACTEC™ System MGIT™ 960
non radioactive

Mycobacteria
Growth
Indicator
Tubes



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Genetic bases of MTC taxonomy

- Various regions of the genome are differently affected by mutations
- House keeping genes, which encode for essential life-function, are little prone to changes and possible codon changes are conservative
- Non encoding regions vary widely
- Halfway are placed the regions encoding for non essential functions and, among the non encoding ones, those which are transcribed

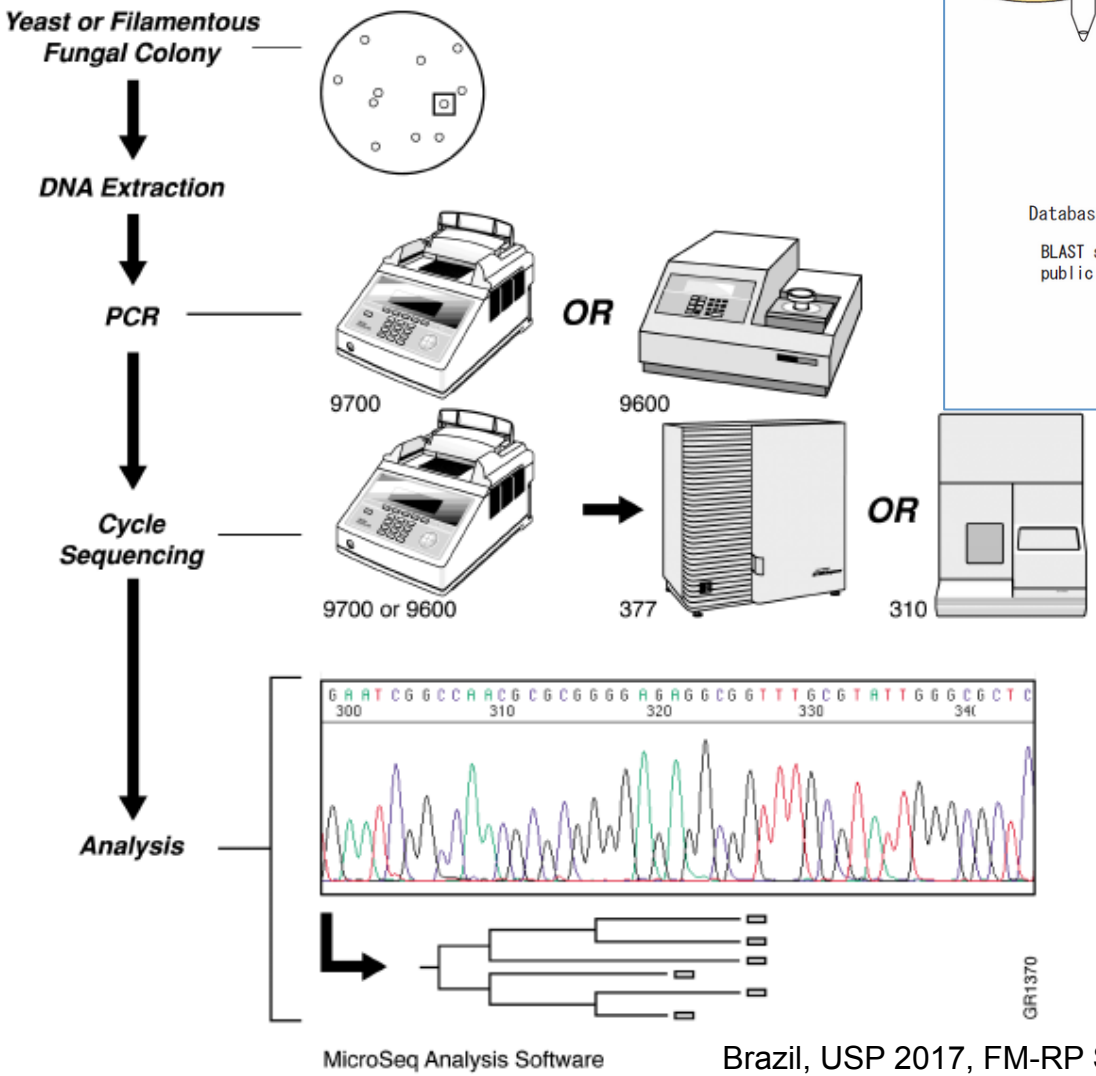
E. Tortoli, 2007

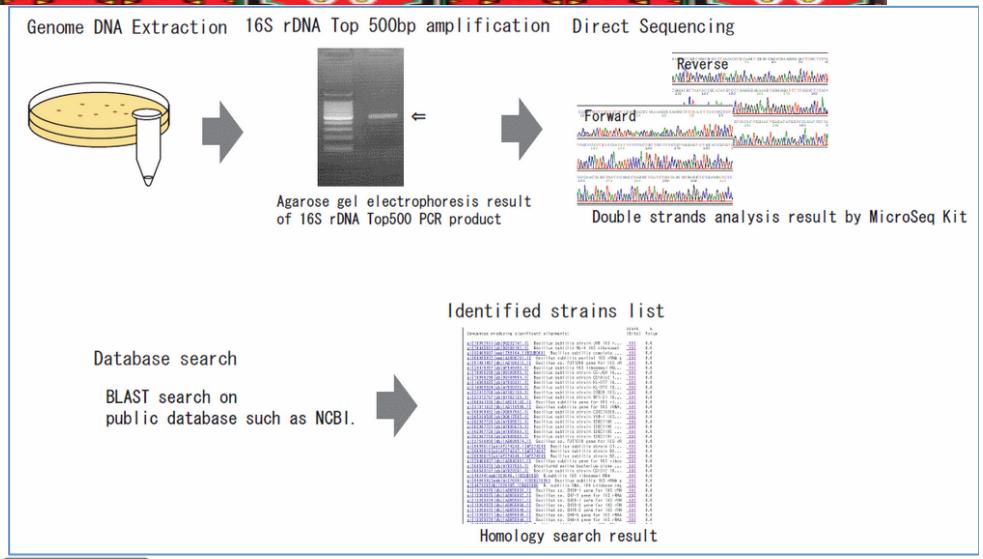
Major taxonomic genetic targets

- 16S rDNA
- 23S rDNA
- *hsp65*
- *recA*
- ITS
- *rpoB*
- *secA*

Tortoli, 2007

16S rRNA sequencing strategy (using Microseq 500 or other)





https://www.hssnet.co.jp/e/2/2_3_6_1.html

Taqman, Cobas, Roche

Molecular Diagnostics of Mycobacterial Infections
Main targets in commercial assays (for culture or direct)

- Amplicor (Roche, PCR) 16S
- AMTDT (GenProbe, Biomérieux, TMA) 16S
- LCx (Abbott, LCR)
- Hain **GenoType Mycobacterium CM VER 2.0** (27 NTM+MTC) inter 16S-23S
- Hain**GenoType Mycobacterium AS VER 1.0** (19NTM) inter 16S-23S
- LAMP (Eiken, Japan) 16S, gyrB

JOURNAL OF CLINICAL MICROBIOLOGY, June 2003, p. 2616–2622 Vol. 41, No. 6
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.6.2616–2622.2003

Loop-Mediated Isothermal Amplification for Direct Detection of *Mycobacterium tuberculosis* Complex, *M. avium*, and *M. intracellulare* in Sputum Samples

Tomotada Iwamoto,[1]* Toshiaki Sonobe,[1] and Kozaburo Hayashi[2]

Department of Bacteriology[1] and Department of Parasitic Agents,[2] Kobe Institute of Health, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan

Received 23 December 2002/Returned for modification 4 February 2003/Accepted 7 March 2003

LAMP method for direct diagnostic

A LAMP reagent kit for detecting the M. tuberculosis complex (Loopamp MTBC detection kit, TB-LAMP; Eiken Chemical, Tokyo, Japan) was launched in **April 2011**. The new reagent features two improvements. First, the test process has been made faster and simpler; by using the kit named the Loopamp PURE DNA extraction kit (Eiken Chemical) for sputum processing, the NALC (N-acetyl-L-cysteine)-NaOH decontamination step is no longer necessary. Second, TB-LAMP is now provided as a dry reagent, allowing easier storage, that is, it can be stored at room temperature with satisfactory shelf life. Figure 1 shows the operation process of PURE and TB-LAMP.

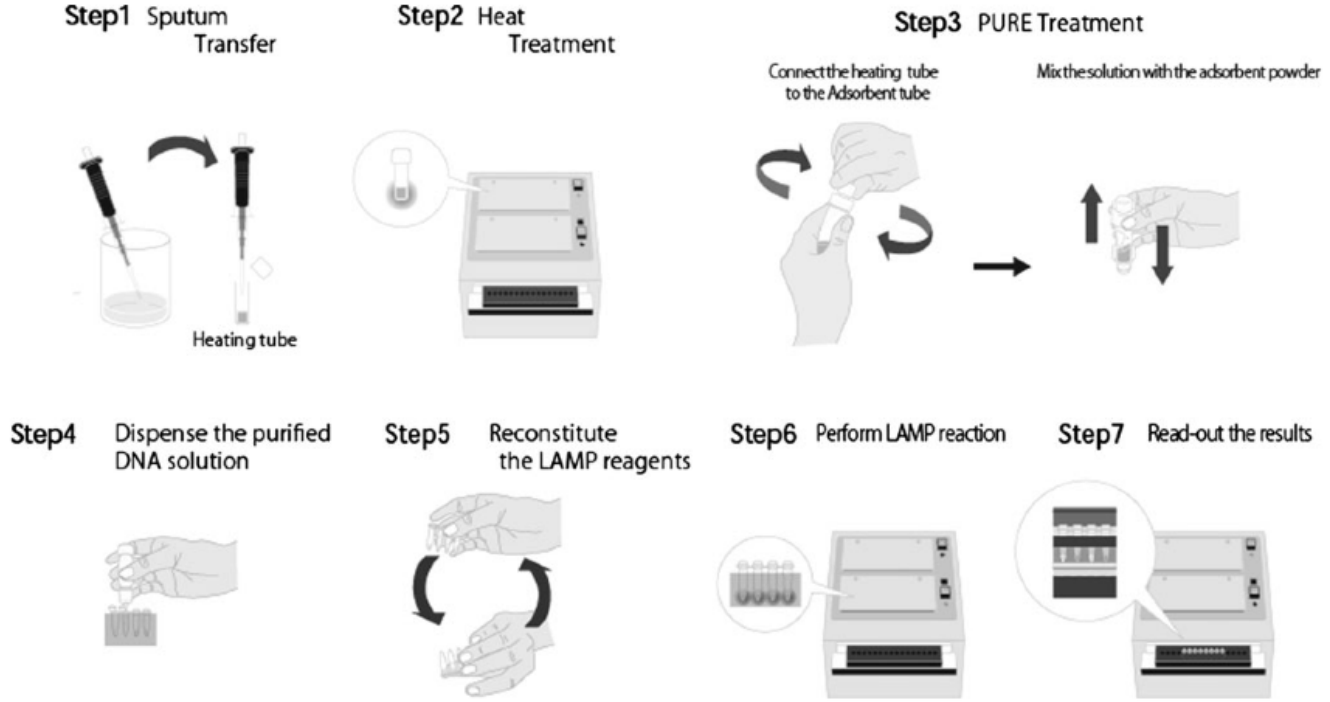


Fig. 1 Diagrams of the process for the PURE-TB-LAMP assay

Y. Mori et al. J Infect Chemother (2013) 19:404–41

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5. Other methods

   Diagnostics
   Molecular Epidemiology

Drug Susceptibility testing

- Classical Agar proportion method
  - « Gold standard »
  - 21 days, tedious, hard work, lot of plates, reading long…

- Liquid medium for drug susceptibility testing
  - 5-6 days
  - Isoniazid, rifampicin, ethambutol and streptomycin
  - Second lines drugs

Alternative to MGIT: ESP Culture System II (AccuMed International, Westlake, Ohio [formerly Difco]

Drug Susceptibility testing

J Clin Microbiol. 2012 Feb; 50(2): 435–440.
PMCID: PMC3264138
doi: 10.1128/JCM.05188-11

Direct Drug Susceptibility Testing of *Mycobacterium tuberculosis* for Rapid Detection of Multidrug Resistance Using the Bactec MGIT 960 System: a Multicenter Study

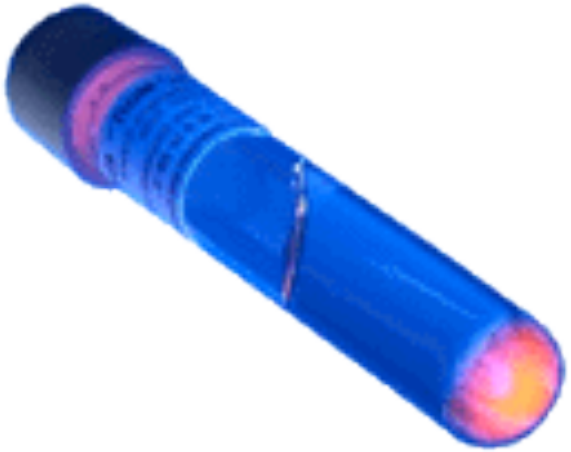
Salman Siddiqi,[a] Altaf Ahmed,[b] Sunil Asif,[b] Digamber Behera,[c] Mona Javaid,[b] Jasmine Jani,[d] Arora Jyoti,[c] Radhika Mahatre,[e] Dewanand Mahto,[d] Elvira Richter,[f] Camilla Rodrigues,[e] Potharaju Visalakshi,[c] and Sabine Rüsch-Gerdes[f]

Phenotypic methods to detect resistance

- Classical method : proportion assay
- Radiometric Bactec system 460TB BD (historical : until 1996)
- **Bactec MGIT960 TB (since 1997)**
- MODS (Caviedes 2000, Moore 2006, Arias 2007)
- FastPlaque assay (Wilson 1997)
- Colorimetric methods (Martin 2007, Palomino 2007, Perkins Cunningham 2007)

BACTEC™ System MGIT™ 960

Mycobacteria
Growth
Indicator
Tubes



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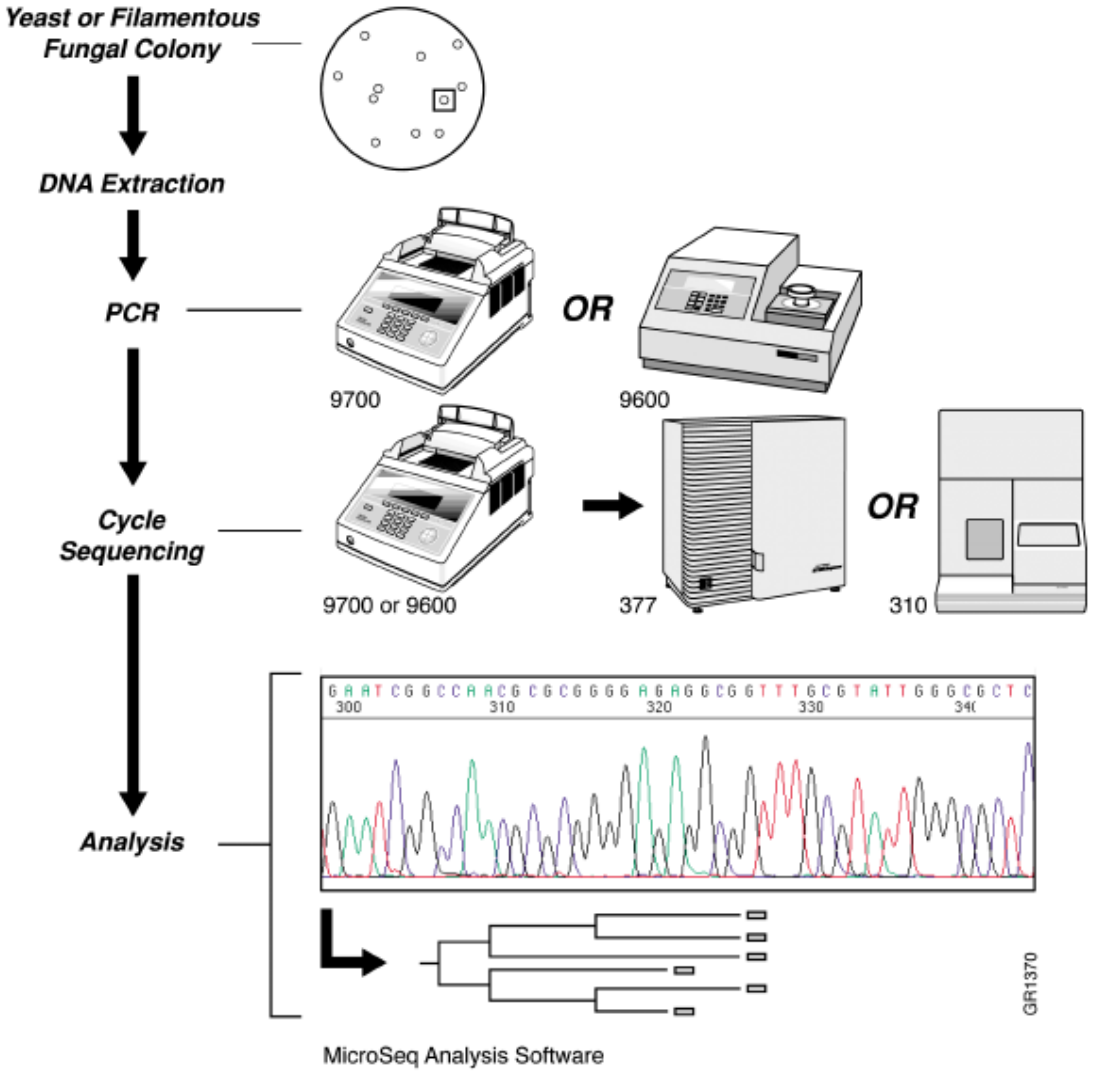
Sequencing for resistance detection



Which genes should we sequence ?

- 1. Rifampicin ----------→ *rpo*B rpoB 516, 526, 531, *170, 491*
- 2. Quinolones----------→ *gyr*A and *gyr*B
- 3. Streptomycin, Kana, Capreo ---→ *rrs*, *rps*L, *eis*
- 4. Pyrazinamide ----------→ *pnc*A
- 5. Isoniazid ----------→ *kat*G (katG315), *inh*A promoter, oxyR-ahpC intergenic region, *mabA*
- 6. Ethambutol ----------→ *emb* *(embB306*
- 7. Other drugs----------→ *bedaquiline, delamanid*

Resistance to rifampin

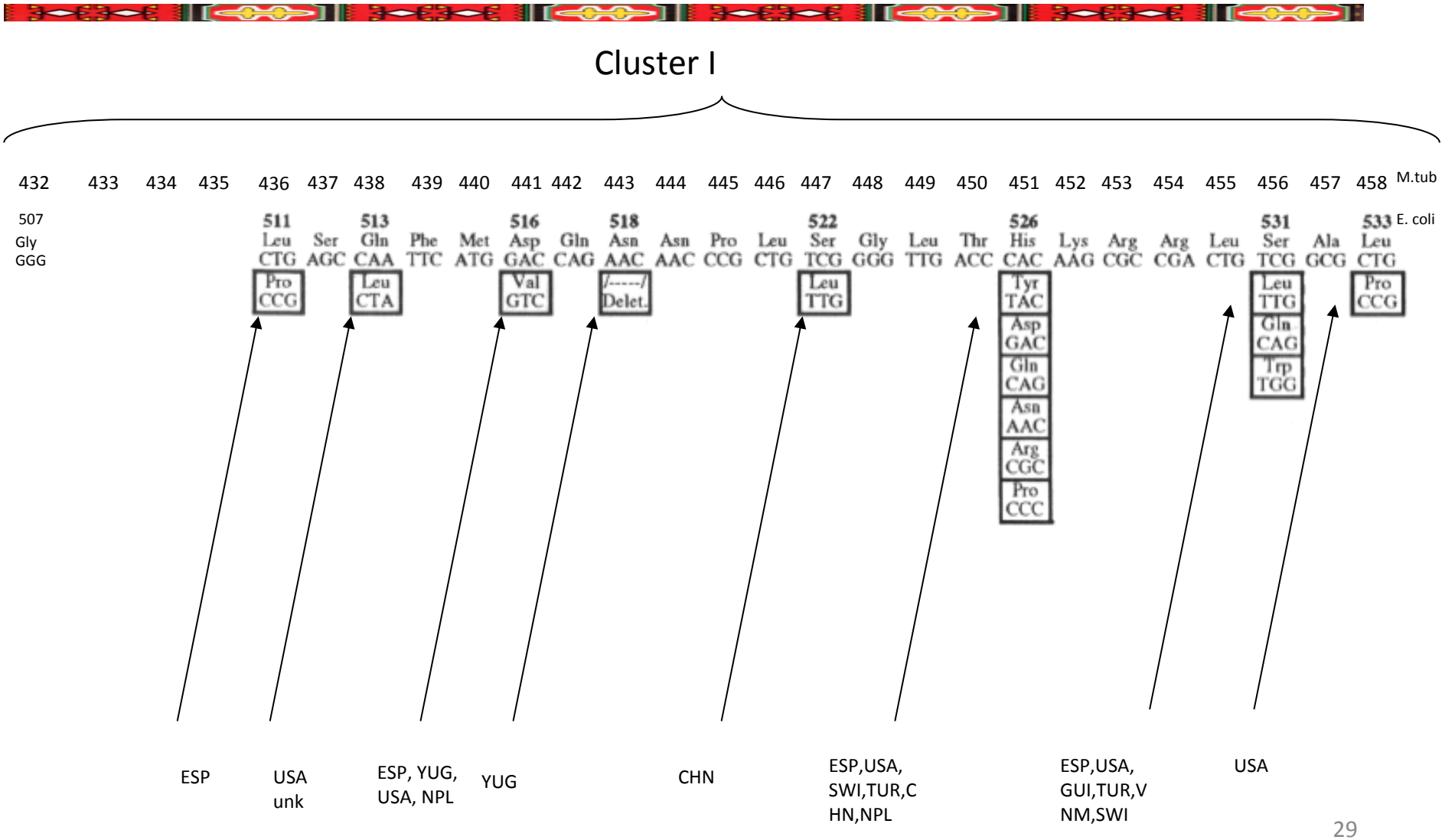
Sequencing of *rpo*B

Primers to be used routinely

- TR1: 5’-tacggtcggcgagcttgatcc-3’
- TR2: 5’-tacggcgtttcgatgaacc-3’
- Length = 410bp

Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*
A. Telenti, P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M.J. Colston, L. Matter K. Schopfer, T. Bodmer
The Lancet 1993 341:647-650

Hot spot for Rif resistance



Codon nomenclature

- Heep, M. B. Brandstätter, U. Rieger, N. Lehn, E. Richter, S. Rüsch-Gerdes and S. Niemann. 2001. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* complex isolates. J. Clin. Microbiol. 39:107-110

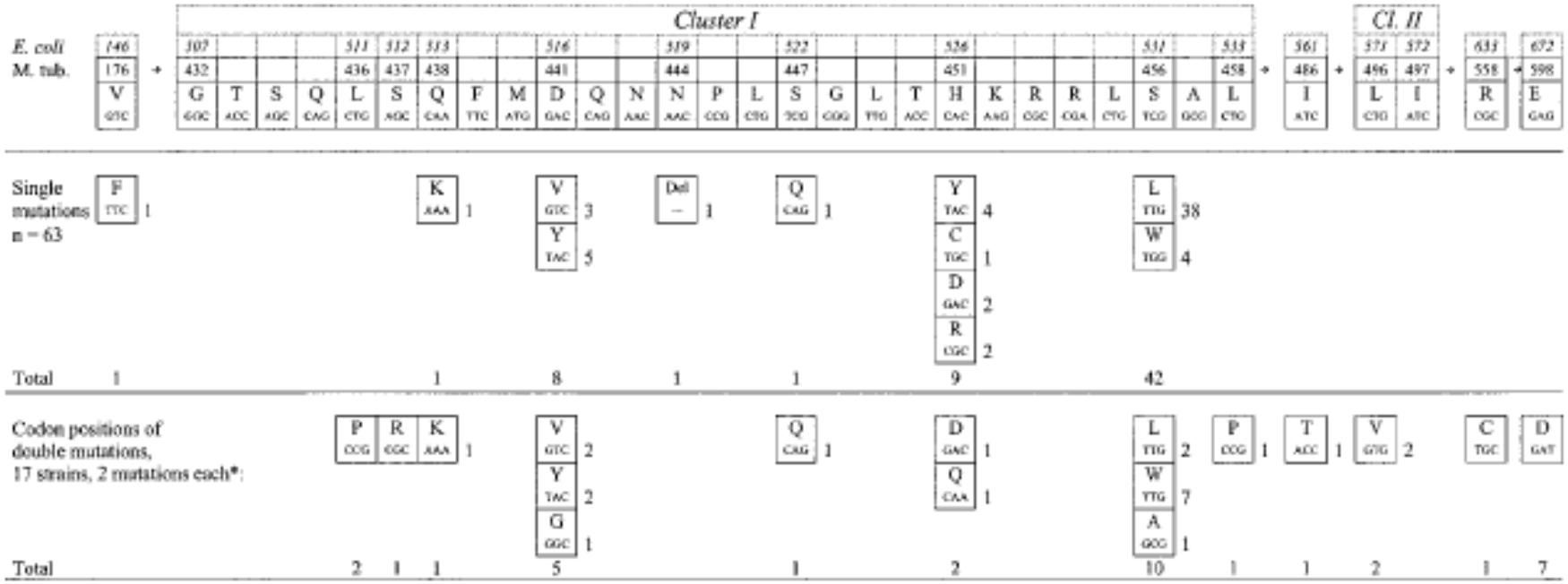


FIG. 1. Frequencies of *rpoB*, single and double mutations associated with RIF resistance in a collection of 80 clinical *M. tuberculosis* isolates from Germany. *, Patterns and numbers of double mutations: L436P plus D441G, one; and L436P plus H451Q, one; S437R plus D441V, one; Q438K plus H451D, one; D441Y plus L496V, two; D441V plus L458P, one; S447Q plus S456A, one; S456L plus I486T, one; S456L plus R558C, one; S456W plus E598D, seven.

Codon nomenclature

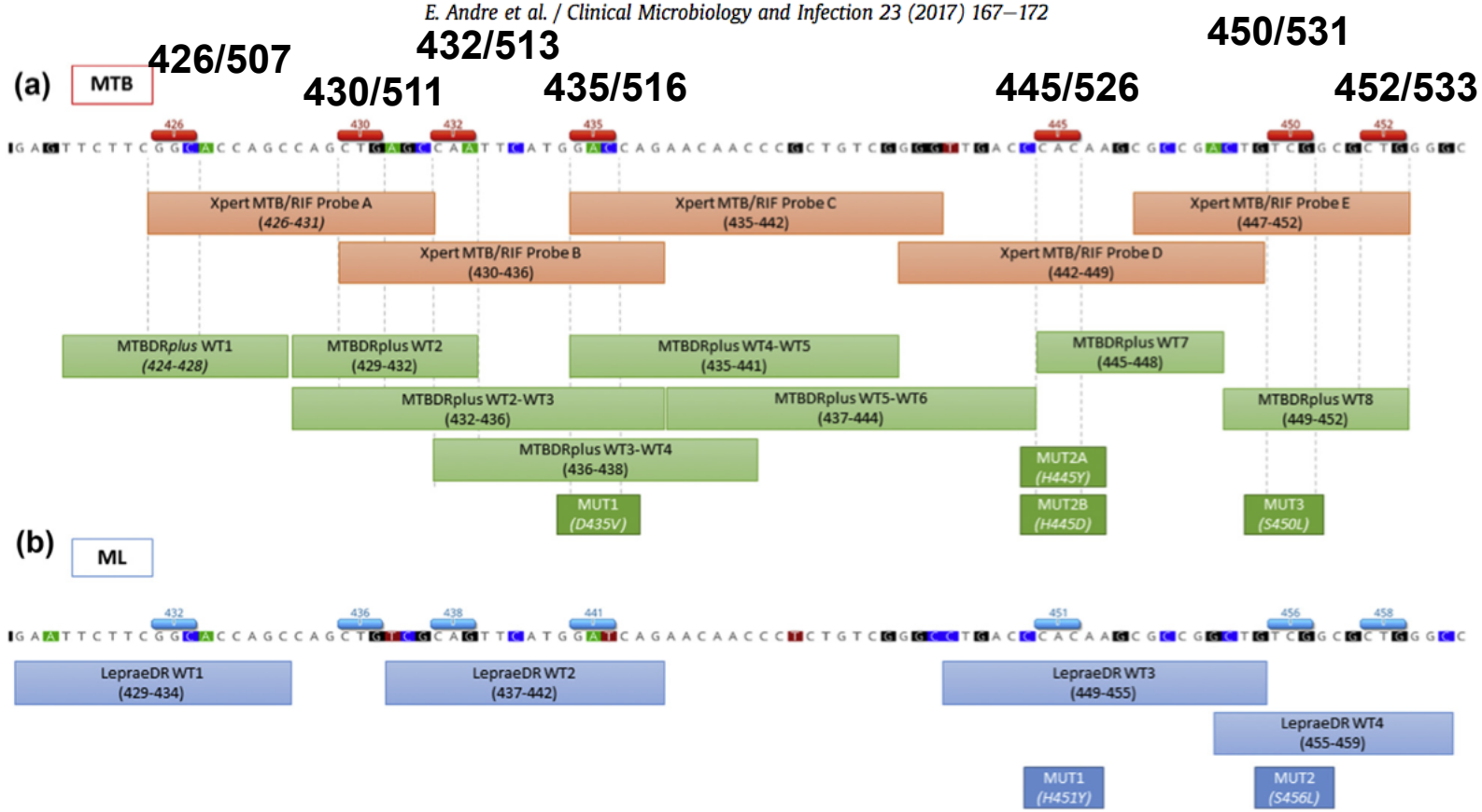


Fig. 2. (a) Alignment of *Mycobacterium tuberculosis* complex rifampicin-resistance-determining region (RRDR) sequence with the targets of Xpert MTB/RIF and the MTBDRplus V2.0 commercial assays. The red bars represent the location of the most common codons associated with rifampicin resistance conferring mutations. The orange bars indicate the regions covered by the five probes of the Xpert MTB/RIF assay. The green bars represent the regions covered by the eight wild-type (WT) and four mutation bands of the GENOTYPE MTBDRPLUS V2.0 assay. (b) Alignment of *Mycobacterium leprae* RRDR sequence with the targets of the LepraeDR commercial assay. The blue bars represent the location of the codons associated with rifampicin resistance conferring mutations, the four WT bands and the two mutation bands. Graph created using GENEIOUS, Biomatters, version 9.1.

Other mutations

- Swaziland, UK, Mozambique, South-Africa
- *rpoB* 170 et 491

Conclusion : commercial assays do not allow satisfactory detection of all mutations. Only WGS will allow full knowledge of the genome.

Troubles in mutation detection : heteroresistance

- « Heteroresistance » : the possibility of mixed-population may in some cases prevent the detection of mutations. In the Heep study, 3 out of 80 RifR isolates did not harbor mutations at first glance. Re-analysis showed that mixed populations prevented the immediate detection of point mutation.

The problem of Heteroresistance in MTB

DJ Operario et al. 2017, PloS ONE

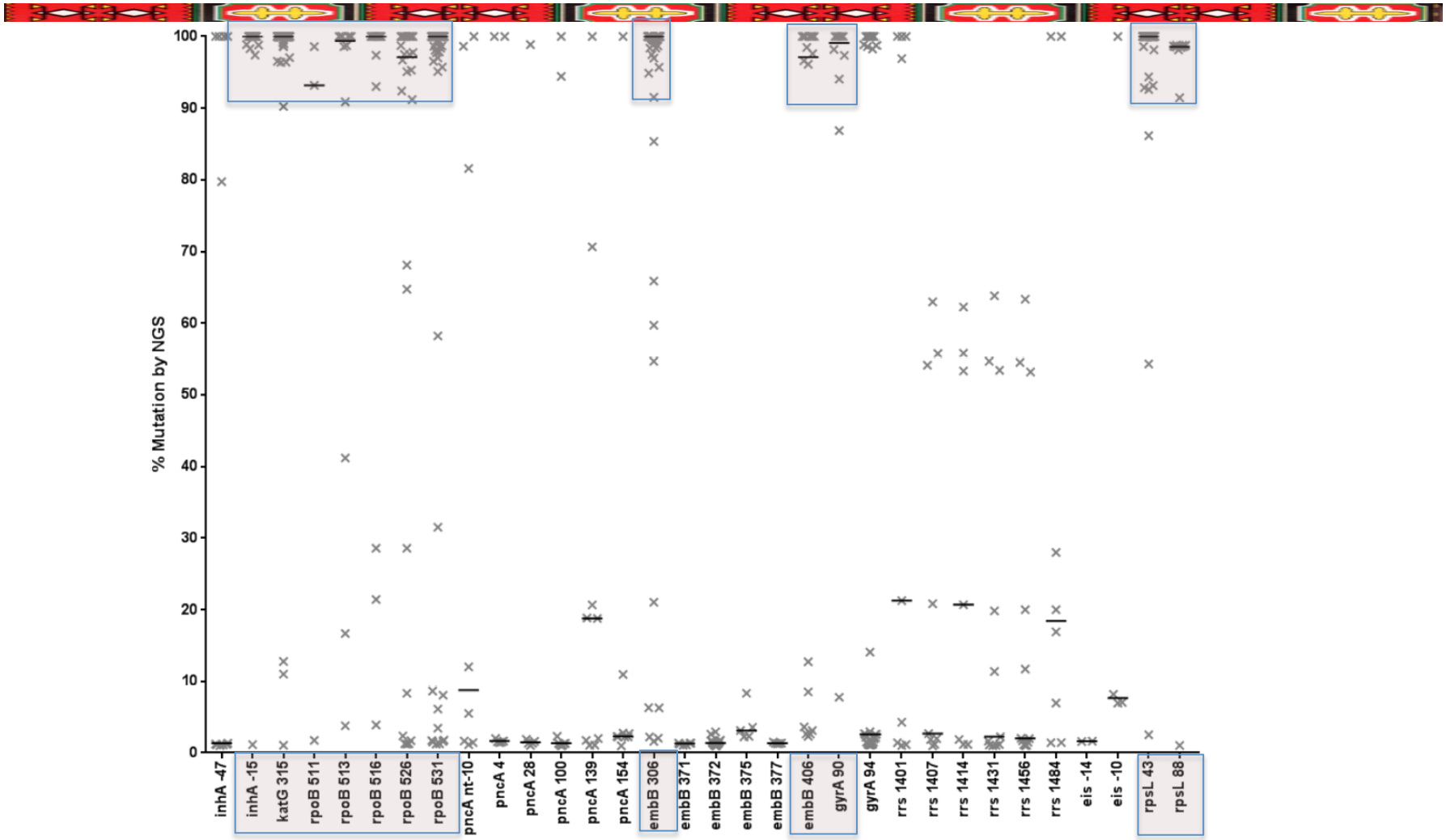


Fig 3. Degree of heteroresistance at major loci. Loci whereby at least 5% of isolates had some degree of mutation are shown, as well as known *rpoB* and *eis* mutations. The y-axis shows the degree of heteroresistance, with each 'X' symbol representing a single isolate. Horizontal lines are median values. Loci are shown on the x axis. In rare instances whereby an isolate had more than one mutation at the same locus, the mutation with the highest degree of heteroresistance is shown.

Resistance to isoniazid

Sequencing of *katG, inhA, ahpc*

Isoniazid resistance

- **katG**

S315R

S315T

- **inhA regulatory region**

C209T

- **ahpC**

720 TELENTI ET AL.

TABLE 1. Primers used in this study

Target	Primer	Product size (bp)
rpoB	TR8, 5′-TGCACGTCGCGGACCTCCA TR9, 5′-TCGCCGCGATCAAGGAGT	157
katG	TB86, 5′-GAAACAGCGGCGCTGGATCGT TB87, 5′-GTTGTCCCATTTCGTCGGGG	209
inhA	TB92, 5′-CCTCGCTGCCCAGAAAGGGA TB93, 5′-ATCCCCCGGTTTCCTCCGGT	248
ahpC	TB90, 5′-CCGATGAGAGCGGTGAGCTG TB91, 5′-ACCACTGCTTTGCCGCCACC	236

Isoniazid resistance

- katG

S315R

S315T

- inhA regulatory region

C209T

- ahpC

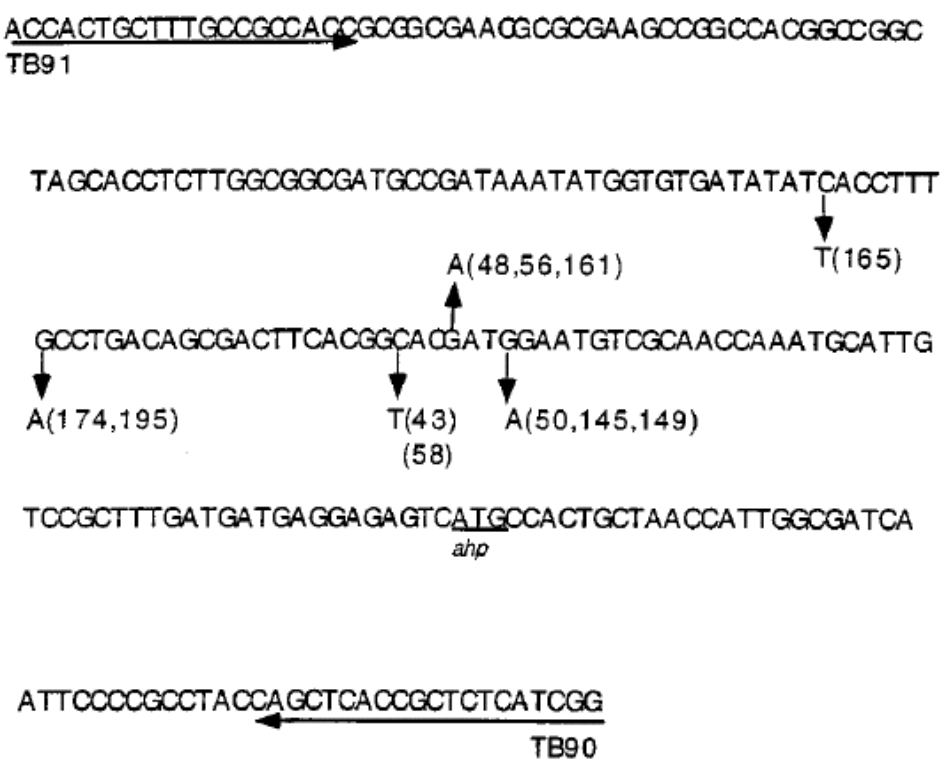


FIG. 1. Nucleotide sequence of the *ahpC-oxyR* intergenic region (GenBank accession number U16243). Indicated are the nucleotide substitutions associated with INH resistance. Numbers indicate the strain identifier.

Resistance to fluoroquinolones

Sequencing of *gyr*A and *gyr*B

Primers to be used routinely

- *gyr*A1: 5’-cagctacatcgactatgcga-3’
- *gyr*A2: 5’-gggcttcggtgtacctcat-3’
- 320 bp fragment
- H37RV Positions 78-397
- *gyr*Bfw: 5’-ccaccgacatcggtggatt-3’
- *gyr*Brev: 5’-ctgccacttgagtttgtaca-3’
- H37Rv Positions 1412-1839
- 429 bp fragment

- *gyr*A: Rv006, 2517bp
- *gyr*B: Rv005, 2145bp
- Subunits of Topoisomerase II

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1994, p. 773–780 Vol. 38, No. 4

Cloning and Nucleotide Sequence of *Mycobacterium tuberculosis* *gyrA* and *gyrB* Genes and Detection of Quinolone Resistance Mutations

HOWARD E. TAKIFF,[1] LEIRIA SALAZAR,[1] CARMEN GUERRERO,[2] WOLFGANG PHILIPP,[3] WAI MUN HUANG,[4] BARRY KREISWIRTH,[5] STEWART T. COLE,[3] WILLIAM R. JACOBS, JR.,[6] AND AMALIO TELENTI[2]*

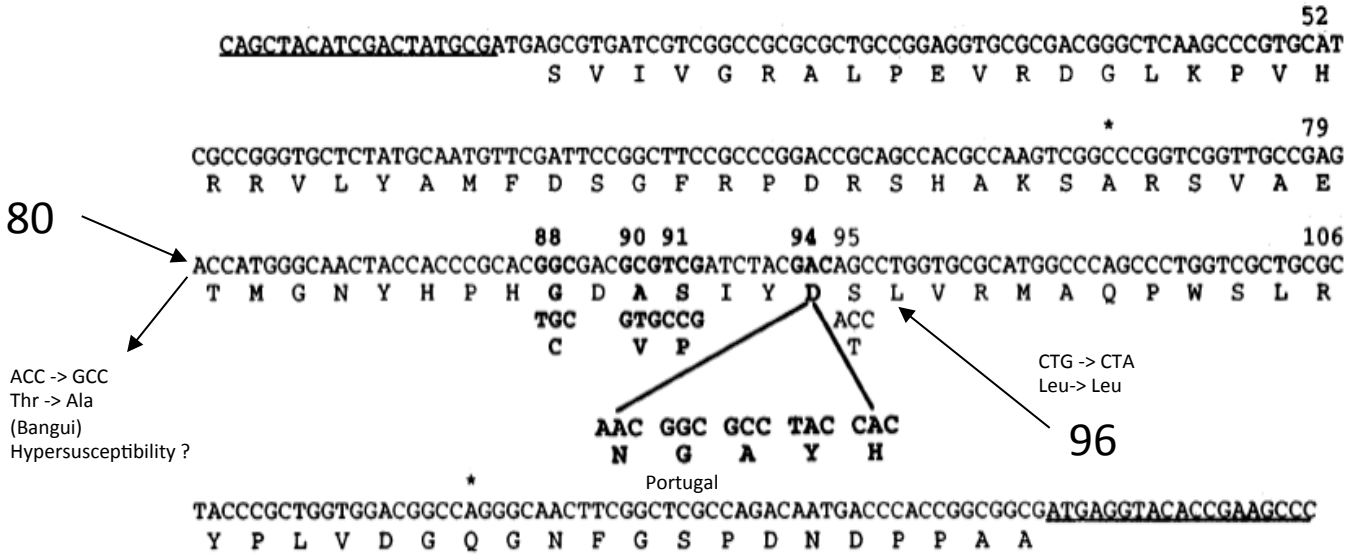


FIG. 4. Nucleotide sequence of the *gyrA* FQ resistance region amplified with primers GyrA1 and GyrA2 (underlined) corresponding to nucleotides 78 to 397 in *M. tuberculosis gyrA*. The deduced amino acid sequence is shown together with mutations in codons 88, 90, 91, and 94 (boldface type), which were found to be associated with ciprofloxacin resistance. Codon 95 may display a serine or a threonine in ciprofloxacin-susceptible strains. An asterisk indicates two additional positions were mutations associated with FQ resistance have been reported in other bacteria. The complete nucleotide sequences of *gyrA* and *gyrB* are deposited in GenBank under accession number L27512.

Resistance to aminoglycosides

Sequencing of *rrs* and *rps*L

Kanamycin, Amikacin, Capreomycin

- Aminoglycosides: Kan, Ami
- Macrocyclic peptide: Cap

-> Cross-resistance

rrs: 16S RNA

tlyA: putative RNA methyl transferase

MICs and sequence for 145 isolates from Georgia (Jugheli et al. 2009)

rrs: A514C, C517C, **A1401G**, **C1402T**, C1443G, T1521C

The four mutants with the C1402T mutations showed high levels of resistance to capreomycin but no resistance to kanamycin and amikacin. Detection of the A1401G mutation appeared to be 100% specific for the detection of resistance to kanamycin and amikacin, while the sensitivities reached 85.9% and 94.2%, respectively.

Primers to be used routinely

- Primers

rpsLfw 5’-GGCCGACAAACAGAACGT-3’
rpsLrev 5’-GTTCACCAACTGGGTGAC-3’
Length = 505 bp

Ribosomal protein S12 =rpsL

rrsfw 5’-GAGAGTTTGATCCTGGCTCAG-3’
rrsrev 5’-TGCACACAGGCCACAAGGGA-3
Length = 1042 bp’

16S rRNA = rrs

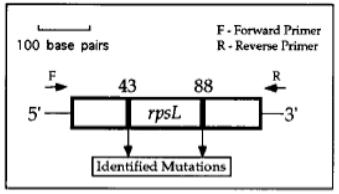
Characterization of *rpsL* and *rrs* Mutations in Streptomycin-Resistant *Mycobacterium tuberculosis* Isolates from Diverse Geographic Localities

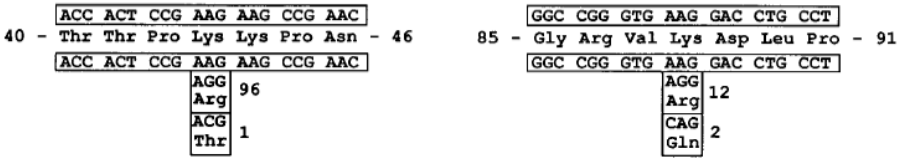
SRINAND SREEVATSAN,[1] XI PAN,[1] KATHRYN E. STOCKBAUER,[1] DIANA L. WILLIAMS,[2] BARRY N. KREISWIRTH,[3] AND JAMES M. MUSSER[1,4]*

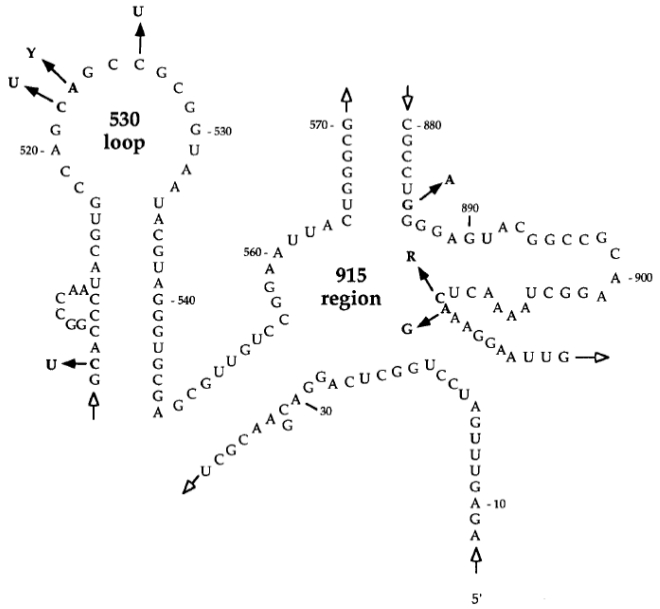
Section of Molecular Pathobiology, Department of Pathology, Baylor College of Medicine,[1] and Clinical Microbiology Laboratory, The Methodist Hospital,[4] Houston Texas 77030; Gillis W. Long Hansen's Disease Center, Louisiana State University, Baton Rouge, Louisiana 70894[2]; and Tuberculosis Laboratory, Public Health Research Institute, New York, New York 10016[3]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1996, p. 1024–1026 Vol. 40, No. 4

rpsL ***rrs***







CLINICAL MICROBIOLOGY REVIEWS, Oct. 1995, p. 496–514
0893-8512/95/$04.00+0

Vol. 8, No. 4

Antimicrobial Agent Resistance in Mycobacteria: Molecular Genetic Insights

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Resistance to Pyrazinamid

Sequencing of *pncA*

Primers to be used routinely

- Primers

pncA-P1 5’-GCTGGTCATGTTCGCGATCG-3’
pncA-P6 5’-GCTTTGCGGCGAGCGCTCCA-3’
Length = 700bp

pyrazinamidase

Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus.

Scorpio A, Zhang Y, Nat Med. 1996 Jun;2(6):662-7
Comment in: Nat Med. 1996 Jun;2(6):635-6

World-wide emergence of XDR Tuberculosis

- Def: MDR TB +3 out of 6 classes of 2nd line drugs
  - Aminoglycosides (amikacin AMK, kanamycin KAN)
  - Polypeptides (capreomycin)
  - Fluoroquinolones (ofloxacin OFX, ciprofloxacin CIP)
  - Thioamides (ethionamide, prothionamide)
  - Cycloserin
  - Para-aminosalicylic acid
- 117 countries in 2016.
- Around 48000 cases worldwide

Reasons of emergence of ultra-resistance

- Non adherence by patients
  - -> Directly Observed treatement of Short-Course (supervision)
- Incorrect drug prescription by prescriptor
  - -> Standardization attempts.
  - ->Improved knowledge of drug functions by MDs
- Incorrect drug supply by providers
  - -> improved international fight against fake drugs
  - -> improved funding for treatment
  - -> discovery of new treatments or new association of drugs
- Incorrect (poor) quality drug
  - -> improved funding of national health systems
- Erratic supply of drug
  - -> susbstitution of National systems by NGO when necessary

Myco TB plate assay

Antimicrob Agents Chemother. 2014 Jan; 58(1): 11–18. PMCID: PMC3910714
doi: 10.1128/AAC.01209-13

Sensititre MYCOTB MIC Plate for Testing *Mycobacterium tuberculosis* Susceptibility to First- and Second-Line Drugs

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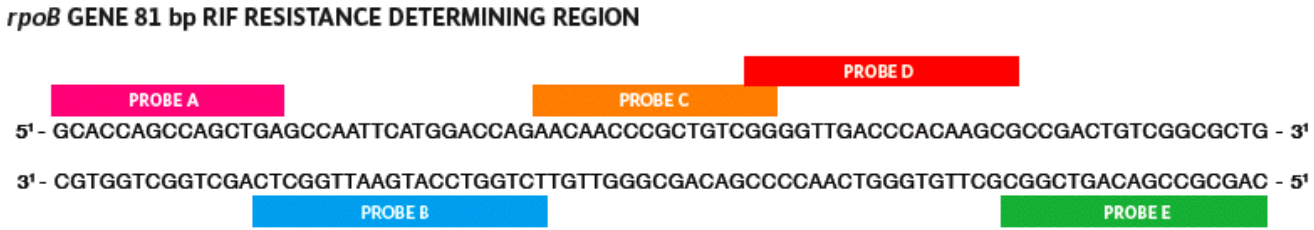
A microtiter plate containing lyophilized antibiotics and configured for determination of MICs to first- and second-line antituberculosis drugs
Median time : 10 days reading

Other Genotypic methods to detect resistance

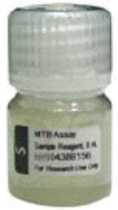
- HAIN Genotype MTBDR plus (Miotto et al. 2008 J Clin Microbiol)
- INNO LIPA RIF TB (Tortoli et al. 2007 Eur J Clin Microbiol Infect Dis)
- RIFOLIGOTYPING (Morcillo et al. 2002 Int J Tuberc Lung Dis)
- SPOLIGORIF, TB-SPRINT, TB-SPRINT ultra, TB-SNPID (Gomgnimbou et al, J Clin Microbiol 2012, J Clin Microbiol 2013)(Bergval et al. 2012, Sengstake et al. 2014)
- CEPHEID/GenXpert (Helb et al. 2010 J Clin Microbiol)



Genotypic methods to detect resistance
« GenExpert and Tuberculosis » 191 ref in Pubmed !

Pour Sample Reagent into sample tube.

Incubate for 15 minutes at room temperature.
(Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)



1

Pipette diluted sample into cartridge.

2

Insert cartridge and start assay.

3

TOTAL HANDS-ON TIME = 2 MINUTES

Xpert® MTB/RIF

PERFORMANCE CHARACTERISTICS OF THE XPERT MTB/RIF ASSAY COMPARED TO DRUG SUSCEPTIBILITY TESTING FOR RIFAMPICIN (RIF)

SENSITIVITY: **96.7%**
SPECIFICITY: **98.6%**
PPV: **93.6%**
NPV: **99.3%**

		DST	
		RIF Resistant	RIF Sensitive
Xpert MTB/RIF	RIF Resistance DETECTED	58	4
	RIF Resistance NOT DETECTED	2	280

TB Laboratory

1. Culture, Phenotypic Identification
2. Molecular identification

   16SrRNA, others
3. Liquid medium for drug susceptibility testing

   BACTEC MGIT 960, other methods
4. Sequencing for resistance detection

   Rifampicin, Isoniazid, quinolones,
   Streptomycin, pyrazinamide, others
5. Other methods

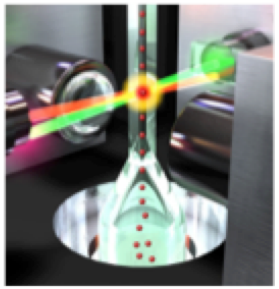
   Diagnostics

   Molecular Epidemiology

xMAP® TECHNOLOGY

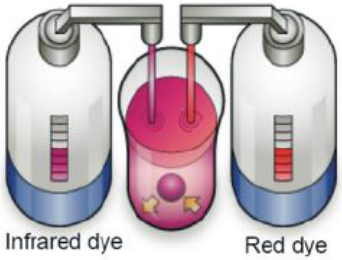
Principle

532 nm
635 nm

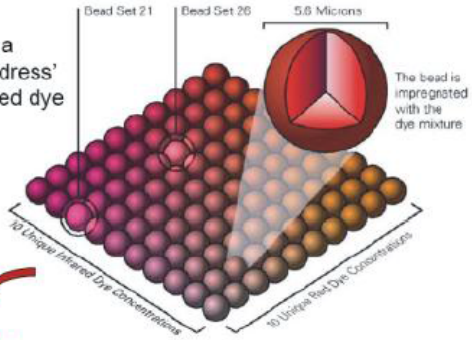


- Red laser: identify microbeads type
- Green Laser vert: quantify fluorescence intensity
- Analysis >100 beads of each type
- Relative fluorescence intensity (RFI)
- For each beads set

Luminex internally color-codes microspheres with precise concentrations of two fluorescent dyes yielding up to 100 distinctly colored bead sets.

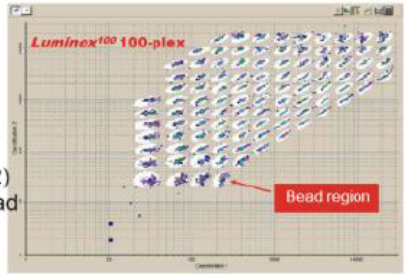


Each bead set has a unique 'spectral address' based on red/infrared dye content

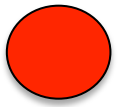


Fluorescence of each bead set is plotted

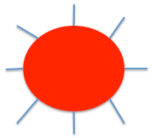
Log plot of red fluorescence intensity (classification 1) vs. infrared fluorescence intensity (classification 2) showing 100 distinct bead regions.



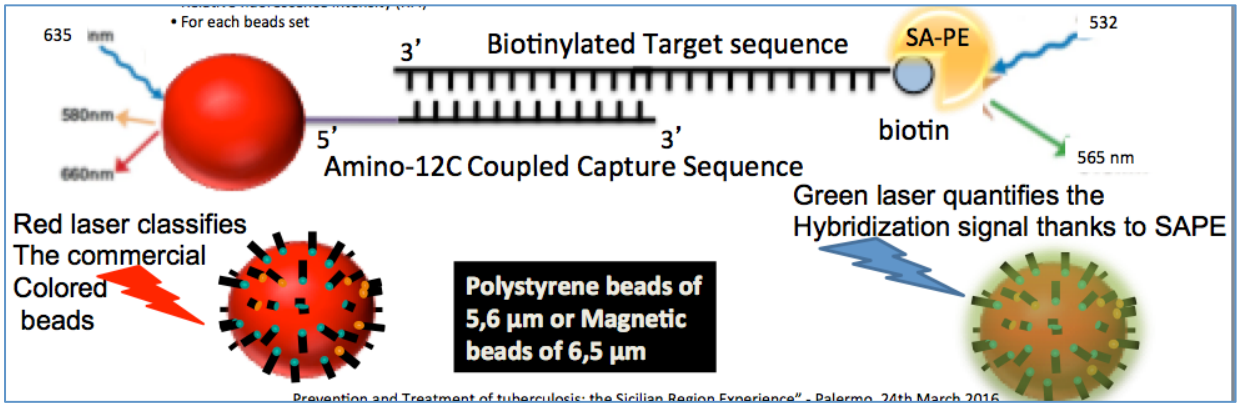
xMAP®



MagPlex®



xTAG®



TB-SPRINT and TB-SNPID Assays Main Characteristics

TB-SPRINT *Dual Priming Oligonucleotide*	**TB-SNPID** *Multiple-Ligation mediated Probe Amplification*
59-Plex (43-Plex+16-Plex)	**50-Plex**
1 day assay (3 hours)	**2 days assay (6 hours)**
PCR **Hybridization** Detection/Reading	RNAse treatment **Hybridization** Ligation **PCR** Detection/Reading
Spoligotyping AND First-lane drug resistance typing	**MTC/NTM Identification on SNP and RDs** **First AND Second lane drug resistance typing (50-Plex)**
43 spacers : spoligotyping **16** drug-resistance markers *rpoB, katG, inhA* (wt **AND** mutations)	**25** genotypic markers, **16** drug-resistance markers (wt **OR** mutations) 1 species-specific marker
Based on Luminex **xMAP®, MagPlex®** Microspheres Produced in France and Distributed by Beamedex®	Based on Luminex proprietary **xTAG®** Microspheres Directly purchased from Luminex, assembled by MRC-Holland and Distributed by Beamedex®

TB-SPRINT : recent publications

1. Gomgnimbou MK, Hernandez-Neuta I, Panaiotov S, Bachiyska E, Palomino JC, Martin A, et al. "TB-SPRINT: TUBERCULOSIS-SPOLIGO-RIFAMPIN-ISONIAZID TYPING"; an All-in-One assay technique for surveillance and control of multi-drug resistant tuberculosis on Luminex® devices. J Clin Microbiol. 2013 Aug 21. [Epub ahead of print];51(11):3527-34.

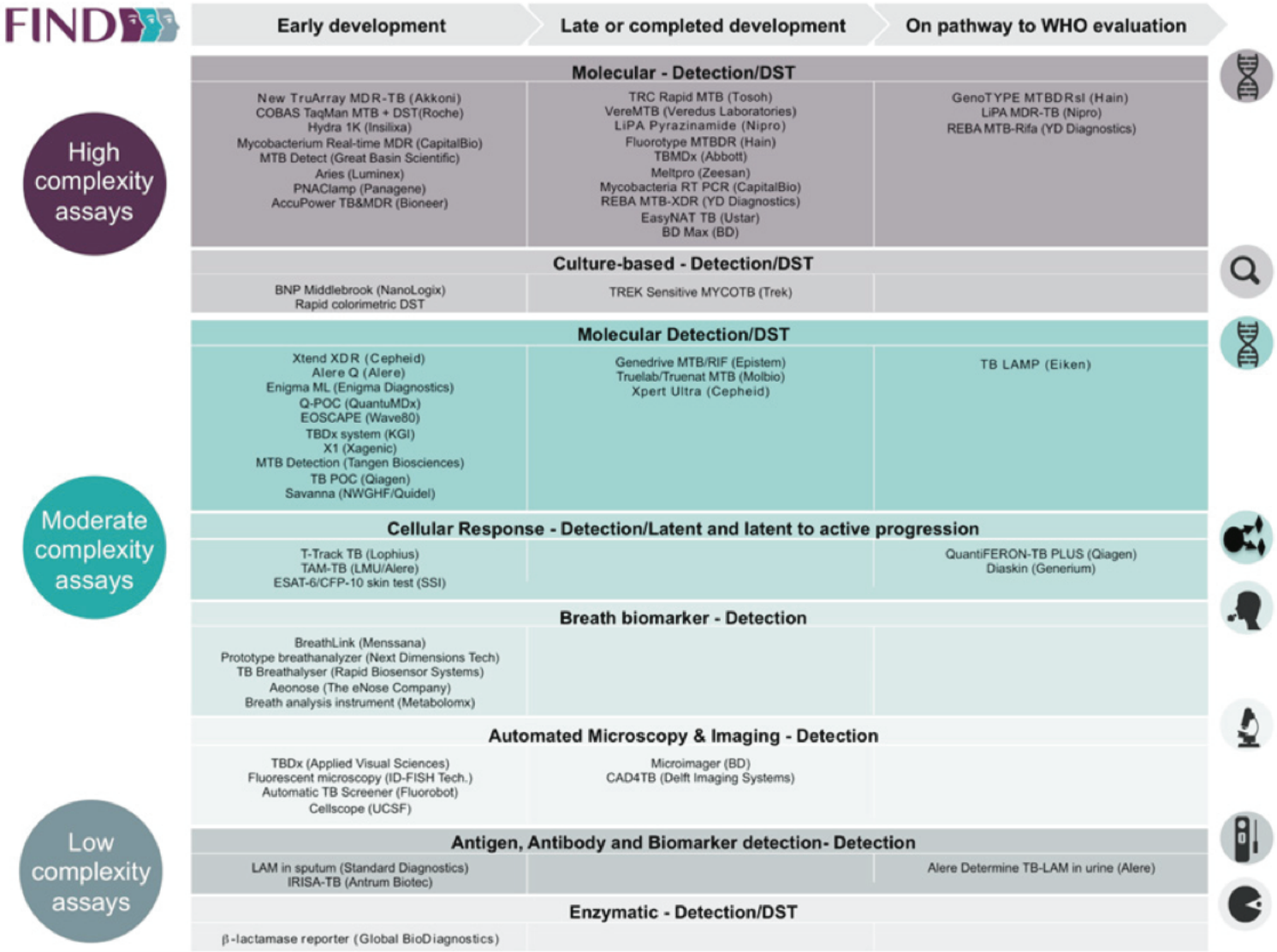
2. Yasmin M, Gomgnimbou MK, Siddiqui RT, Refregier G, Sola C. Multi-drug resistant Mycobacterium tuberculosis complex genetic diversity and clues on recent transmission in Punjab, Pakistan. Infect Genet Evol. 2014 Jun 27;27C: 6-14.

3. Dantas N, Suffys P, da Silva Carvalho W, Gomes HM, Neves de Almeida I, Jouca de Assis L, et al. Multidrug-resistant Mycobacterium tuberculosis Genetic Diversity in Minas Gerais state, Brazil. BMC Infectious Diseases. 2015;15:306.

4. Molina-Moya B, Gomgnimbou MK, Lafoz C, Lacoma A, Prat C, Refregier G, et al. Molecular Characterization of Mycobacterium tuberculosis Strains with TB-SPRINT. The American journal of tropical medicine and hygiene. 2017 Jul 10.

Innovations are to be expected

Figure 4. Current FIND TB diagnostics pipeline listing the development phases and the types of technologies in development or evaluation



Source: FIND, Geneva.

Whole-Genome Sequencing/Next-Gen Sequencing

- Still in its infancy
- « *Complexity made simple* » is a difficult task
- Standardization of Molecular Biology procedures, Standardization of Algorithms and Bioinformatical procedures, will be difficult to achieve…
- Lot of data produced, but onoy few results.
- *Technoscience* more important than the goal to achieve… : many financial interest…lobbies and power
- But : true innovation (exemple : Oxford Nanopore sequencing…) is expected to promote changes.

Whole-Genome Sequencing/Next-Gen Sequencing

PhyResSE: a Web Tool Delineating *Mycobacterium tuberculosis* Antibiotic Resistance and Lineage from Whole-Genome Sequencing Data

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Feuerriegel S et al. J Clin Microbiol. 2015 Jun;53(6):1908-14.

History of Molecular Epidemiology of tuberculosis

- Phage typing
- IS6110-RFLP
- PFGE
- Spoligotyping
- YATM (Yet another typing method)
- MIRU-VNTR typing (MLVA)
- MLST
- SNPs typing
- Whole Genome Sequencing/Next generation Sequencing
- cgMLST
- Multiplexed SNP-typing on microbeads (TB-SPRINT, TB-RINT, TB-ULTRA, PZA-Typing, Beijing-typing, etc…)