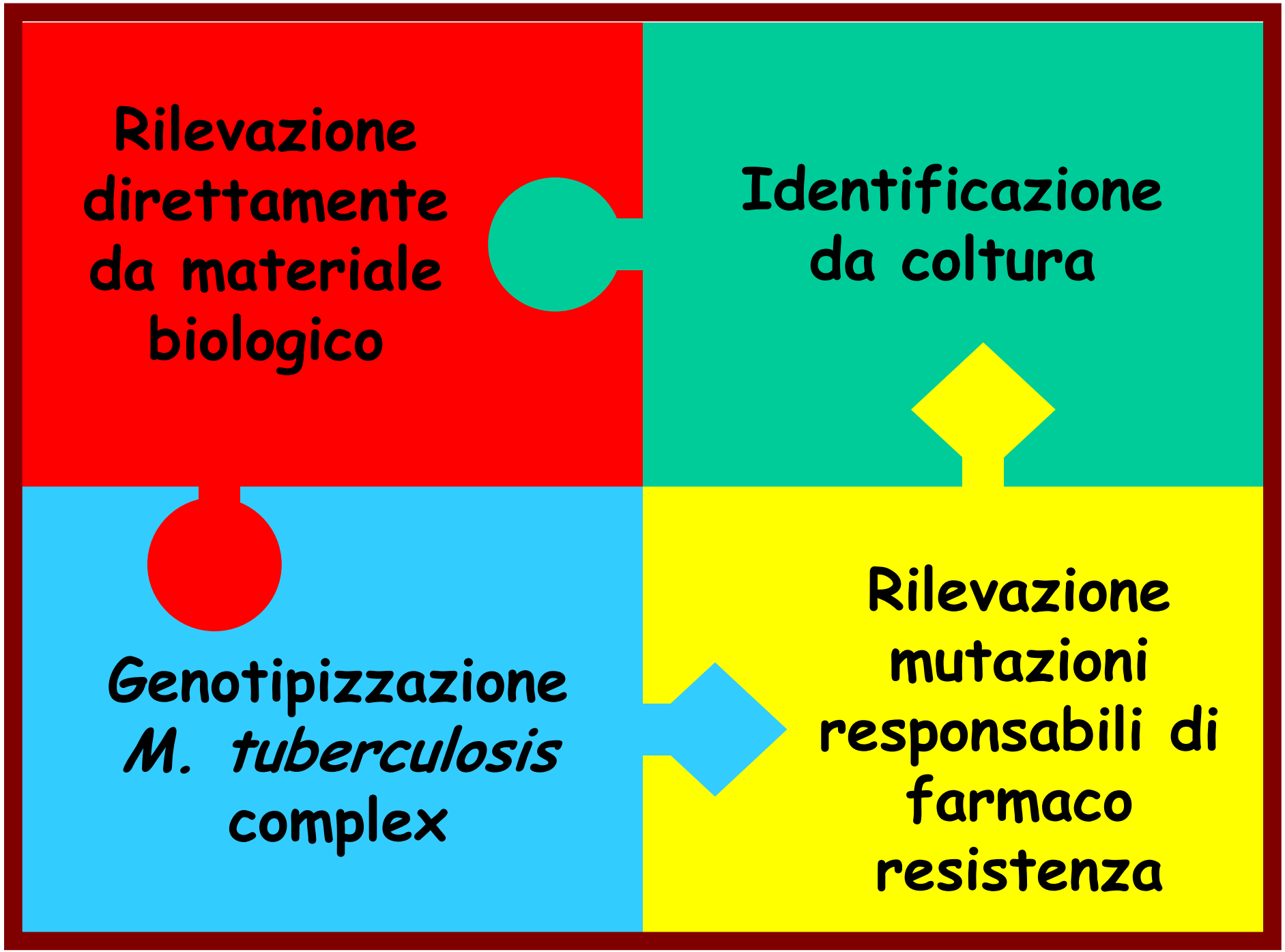
1° Congresso NEWMICRO
Biotecnologie e diagnostica molecolare nella diagnosi delle malattie infettive

Trento, 20 Gennaio 2011

NEWMICRO
Network di Microbiologia e Virologia del Nord Est

Le tecniche molecolari nella diagnosi delle infezioni da micobatteri

Claudio Scarparo
S.O.C. di Microbiologia
Azienda Ospedaliero Universitaria S. Maria della Misericordia - Udine


Rilevazione direttamente da materiale biologico
Identificazione da coltura
Genotipizzazione *M. tuberculosis* complex
Rilevazione mutazioni responsabili di farmaco resistenza

I test di amplificazione diretta (DAT)

consentono il simultaneo rilevamento ed identificazione del *M. tuberculosis* complex direttamente dai campioni clinici, con risultati disponibili nel giro di poche ore.

Raccomandazioni dei *CDC* sull'impiego del test di amplificazione

MMWR 2009; 58: 7-10

- NAA testing should be performed **on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB** for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.
- **Culture remains the gold standard** for laboratory confirmation of TB and is required for isolating bacteria for drug-susceptibility testing and genotyping.
- Although NAA testing is recommended to aid in the initial diagnosis of persons suspected to have TB, the **currently available NAA tests should not be ordered routinely when the clinical suspicion of TB is low, because the positive predictive value of the NAA test is <50% for such cases**

American Thoracic Society Documents

American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: Controlling Tuberculosis in the United States

This Official Joint Statement of the American Thoracic Society, the Centers for Disease Control and Prevention, and the Infectious Diseases Society of America was approved by the ATS Board of Directors, June 2004, the Centers for Disease Control and Prevention, November 2004, and the IDSA Board of Directors, March 2005.

Am J Respir Crit Care Med 2005; 172: 1169-1227

Tempi di refertazione

TABLE 3. ESSENTIAL LABORATORY TESTS FOR TUBERCULOSIS CONTROL

Test	Maximum Turnaround Time
Microscopy for acid-fast bacilli	≤ 24 h from specimen collection or, if test is performed offsite, ≤ 24 h from receipt in laboratory; if latter, time from specimen collection to laboratory receipt should be ≤ 24 h
Nucleic acid amplification assay	≤ 48 h from date of specimen collection
Mycobacterial growth detection by culture	≤ 14 d from date of specimen collection
Identification of cultured mycobacteria	≤ 21 d from date of specimen collection
Drug susceptibility testing	≤ 30 d from date of specimen collection
Drug susceptibility testing of second-line drugs	≤ 4 wk from date of request

I sistemi commerciali (1)

Sistema	E-MTD	Amplicor	ProbeTec
Metodo	TMA	PCR	SDA
Bersaglio	rRNA	rDNA	IS 6110
Lettura	Chemiolumi-nescenza	Colorimetria	Fluorimetria
Automazione	Assente	Lettura	Amplificazione e lettura
C. I. A.	No	Si	Si
Licenza FDA	Sì	Sì	No

La PCR Real-Time

- La PCR *real-time* (*qRT-PCR)* presenta indiscutibili vantaggi rispetto alla *cnPCR*:
  - Rapidità
  - Ampio *range* di quantificazione del *target*
  - Riduzione della contaminazione da *amplicons*
  - Alta sensibilità intesa come più basso limite di *detection*

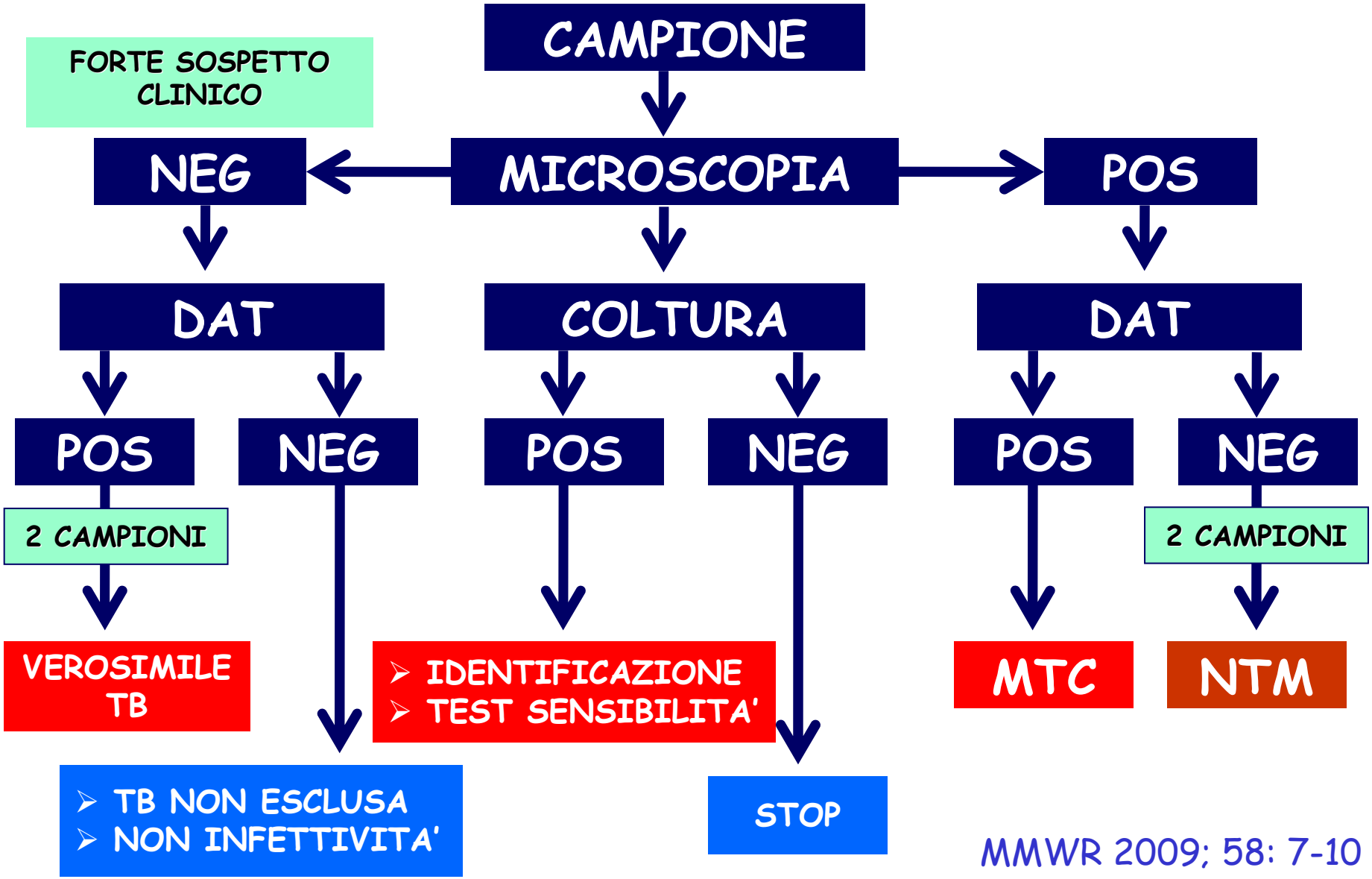
I sistemi commerciali (2)

Sistema	Real Art MTB PCR	MTB Q PCR Alert	COBAS TaqMan	DUPLICa MTB
Metodo	qRT-PCR	RT-PCR	RT-PCR	RT-PCR
Target	16S rDNA	IS 6110	16S rDNA	16S-23S rDNA
Sonda	TaqMan	TaqMan	TaqMan	TaqMan
C.I.A.	eterologo	eterologo	omologo	omologo
Estrazione	Lisi-cattura	Lisi-cattura	Lisi	Lisi-cattura
Sensibilità (n. di copie)	1	10	5	10
Campioni	R	R + E	R	R + E

Uso autorizzato (FDA)

- Campioni respiratori
  - Contemporanea esecuzione di microscopia e coltura
  - Raccolti da pazienti non trattati
  - Microscopia positiva per BAAR
  - Microscopia negativa per BAAR in caso di forte sospetto clinico

Algoritmo interpretativo



MMWR 2009; 58: 7-10

Uso accessorio

- Campioni extrarespiratori
  - Risultati più affidabili con:
    - Microscopia positiva
    - Aspirati gastrici, pus, biopsie
  - Risultati meno affidabili con:
    - Microscopia negativa
    - Liquor o altri liquidi sierosi

Indicazioni

- Impiego mirato sulla base di un congruo sospetto clinico
  - ***esperienza clinica***
  - di norma sul primo di tre campioni
  - **servono a confermare la TB (ruling in)**
  - **NON servono ad escluderla (ruling out)**

- La resa diagnostica del test dipende in larga misura da:
  - qualità del campione
  - appropriatezza della sede di prelievo

Selezione dei pazienti

- Indicatori predittivi di TB polmonare
  - Immigrazione
  - Infezione da HIV
  - Dimagrimento
  - Sudorazioni notturne
  - Lesioni polmonari
  - ✓ cavitarie
  - ✓ in sede apicale

Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis

Greco S, et al. Thorax 2006;61:783-790

Le metanalisi (campioni respiratori)

Table 1 Pooled values* (95% confidence intervals) of diagnostic odds ratio (DOR), sensitivity, and specificity of five commercial nucleic acid amplification tests (NAATs)

Test	NAA method	AFB+ DOR	AFB+ Sensitivity	AFB+ Specificity	AFB− DOR	AFB− Sensitivity	AFB− Specificity
Amplicor	PCR	117 (56 to 246)	0.96 (0.94 to 0.97)	0.83 (0.8 to 0.86)	77 (51 to 115)	0.61 (0.57 to 0.65)	0.97 (0.968 to 0.974)
Cobas Amplicor	PCR	99 (56 to 173)	0.96 (0.95 to 0.97)	0.74 (0.68 to 0.8)	220 (144 to 335)	0.64 (0.59 to 0.69)	0.993 (0.992 to 0,994)
BDP	SDA	181 (39 to 834)	0.98 (0.96 to 0.99)	0.89 (0.84 to 0.93)	96 (53 to 175)	0.71 (0.66 to 0.76)	0.97 (0.964 to 0.974)
E-MTD	TMA	314 (99 to 995)	0.97 (0.95 to 0.98)	0.96 (0.93 to 0.97)	157 (48 to 510)	0.76 (0.7 to 0.8)	0.97 (0.966 to 0.974)
LCx	LCR	42 (12 to 142)	0.96 (0.94 to 0.98)	0.71 (0.64 to 0.78)	71 (38 to 132)	0.57 (0.5 to 0.64)	0.98 (0.978 to 0.985)

PCR, polymerase chain reaction; SDA, strand displacement amplification; TMA, trascription mediated amplification; LCR, ligase chain reaction; DOR, diagnostic odds ratio.
*Random effect model.

Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis

Pai M, et al. Lancet Infect Dis 2003; 3: 633-43

Le metanalisi (Liquor cefalorachidiano)

Table 3. Summary measures of test accuracy for all studies, commercial, and in-house tests

Test property	Summary measure of test accuracy* (95% CI)	Test for heterogeneity† p value
All studies (n=49)		
Sensitivity	0·71 (0·63, 0·77)	<0·001
Specificity	0·95 (0·92, 0·97)	<0·001
Positive likelihood ratio (LR+)	15·4 (9·6, 24·9)	<0·001
Negative likelihood ratio (LR–)	0·25 (0·15, 0·39)	<0·001
Diagnostic odds ratio (DOR)	59·4 (40·6, 86·9)	0·43
Commercial tests (n=14)		
Sensitivity	0·56 (0·46, 0·66)	0·10
Specificity	0·98 (0·97, 0·99)	0·10
Positive likelihood ratio (LR+)	35·1 (19·0, 64·6)	0·78
Negative likelihood ratio (LR–)	0·44 (0·33, 0·60)	0·07
Diagnostic odds ratio (DOR)	96·4 (42·8, 217·3)	0·75
In-house tests (n=35)		
Sensitivity	0·76 (0·67, 0·83)	<0·001
Specificity	0·92 (0·88, 0·95)	<0·001
Positive likelihood ratio (LR+)	11·5 (6·8, 19·7)	<0·001
Negative likelihood ratio (LR–)	0·21 (0·11, 0·40)	<0·001
Diagnostic odds ratio (DOR)	54·8 (34·4, 87·2)	0·28

*Random effects model. †χ^2 test for heterogeneity. CI=confidence interval

Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis

Le metanalisi (Liquidi pleurici)

Pai M, et al. BMC Infect Dis. 2004 Feb 23;4:6. Review.

Measure of test accuracy	Pooled summary measure[a] (95% CI)	Test for heterogeneity[b] p value
Amplicor [N = 4]		
Sensitivity	0.37(0.24, 0.52)	0.18
Specificity	0.99 (0.97, 1.0)	0.12
Positive Likelihood Ratio (LR+)	52.8 (11.8, 236.2)	0.14
Negative likelihood Ratio (LR-)	0.59 (0.37, 0.95)	0.20
Diagnostic odds ratio (DOR)	105.5 (15.1, 733.9)	0.10
LCx [N = 4]		
Sensitivity	0.72 (0.54, 0.84)	0.12
Specificity	0.97 (0.95, 0.98)	0.53
Positive Likelihood Ratio (LR+)	25.6 (14.1, 46.4)	0.57
Negative likelihood Ratio (LR-)	0.32 (0.14, 0.68)	0.09
Diagnostic odds ratio (DOR)	93.2 (35.6, 243.7)	0.38
MTD [N = 6]		
Sensitivity	0.77 (0.62, 0.88)	<0.001
Specificity	0.98 (0.96, 0.99)	0.66
Positive Likelihood Ratio (LR+)	17.4 (6.1, 49.7)	0.26
Negative likelihood Ratio (LR-)	0.31 (0.09, 1.03)	<0.001
Diagnostic odds ratio (DOR)	72.9 (9.9, 533.4)	0.03

Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis

Le metanalisi (Liquidi pleurici)

Pai M, et al. BMC Infect Dis. 2004 Feb 23;4:6. Review

Measure of test accuracy	Pooled summary measure[a] (95% CI)	Test for heterogeneity[b] p value
All Commercial Tests [N = 14]		
Sensitivity	0.62 (0.43, 0.77)	<0.001
Specificity	0.98 (0.96, 0.98)	0.12
Positive Likelihood Ratio (LR+)	25.4 (16.2, 40.0)	0.46
Negative likelihood Ratio (LR-)	0.40 (0.24, 0.67)	<0.001
Diagnostic odds ratio (DOR)	80.9 (34.4, 190.4)	0.09
In-house Tests [N = 26]		
Sensitivity	0.71 (0.63, 0.78)	<0.001
Specificity	0.93 (0.88, 0.96)	<0.001
Positive Likelihood Ratio (LR+)	9.7 (5.7, 16.6)	<0.001
Negative likelihood Ratio (LR-)	0.32 (0.23, 0.43)	<0.001
Diagnostic odds ratio (DOR)	42.4 (22.2, 81.1)	<0.001

OPEN ACCESS Freely available online

PLoS one

Commercial Nucleic-Acid Amplification Tests for Diagnosis of Pulmonary Tuberculosis in Respiratory Specimens: Meta-Analysis and Meta-Regression

Daphne I. Ling[1], Laura L. Flores[2], Lee W. Riley[1,3], Madhukar Pai[4]*

February 2008 | Issue 2 | e1536

1 Division of Epidemiology, School of Public Health, University of California, Berkeley, California, United States of America, 2 Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, San Francisco, California, United States of America, 3 Division of Infectious Diseases, School of Public Health, University of California, Berkeley, California, United States of America, 4 Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Quebec, Canada

Table 3. Pooled Summary Estimates of 125 Commercial NAAT Studies (adding 0.5 to all cells of studies with 0 values)

Accuracy Measure	Accuracy Estimate (95% Confidence Interval)	Chi^2 test of heterogeneity	P value for heterogeneity
Sensitivity	0.85 (0.847, 0.86)	1121.69	<.001
Specificity	0.968 (0.967, 0.969)	3748.64	<.001
Positive Likelihood Ratio (LR+)	32.74 (26.01, 41.22)	3831.86	<.001
Negative Likelihood Ratio (LR-)	0.14 (0.12, 0.16)	1495.00	<.001
Diagnostic Odds Ratio (DOR)	268.88 (212.07, 340.9)	869.46	<.001

Ling DI et al., PloS ONE, February 2008, Issue 2. e1536

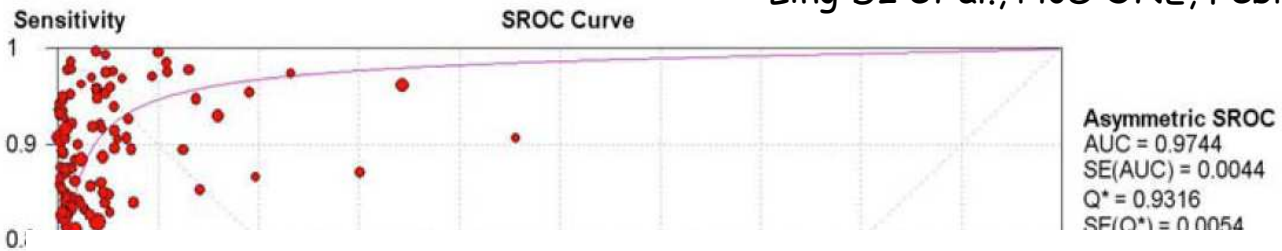


Table 5. Likelihood Ratios Stratified by Commerical NAAT

Test	Positive Likelihood Ratio (95% CI)	P value for heterogeneity	Negative Likelihood Ratio (95% CI)	P value for heterogeneity
Amplicor	26.04 (17.04, 39.80)	<.001	0.15 (0.11, 0.22)	<.001
Cobas Amplicor	58.59 (37.77, 90.86)	<.001	0.17 (0.13, 0.22)	<.001
AMTD	28.75 (17.79, 46.47)	<.001	0.12 (0.09, 0.17)	<.001
E-AMTD	57.55 (25.49, 129.92)	<.001	0.12 (0.07, 0.22)	<.001
LCx	26.91 (17.21, 42.09)	<.001	0.16 (0.12, 0.20)	<.001
BD-ProbeTec	20.11 (10.42, 38.82)	<.001	0.06 (0.04, 0.10)	0.264
BD-ProbeTec-ET	37.07 (19.18, 71.65)	<.001	0.14 (0.09, 0.20)	0.002

Figure 4. SROC plot with best-fitting asymmetric curve. Each solid circle represents each study in the meta-analysis. The curve is the regression line that summarizes the overall diagnostic accuracy. SROC = summary receiver operating characteristic; AUC = area under the curve; SE(AUC) = standard error of AUC; Q* = an index defined by the point on the SROC curve where the sensitivity and specificity are equal, which is the point closest to the top-left corner of the ROC space; SE(Q*) = standard error of Q* index.
doi:10.1371/journal.pone.0001536.g004

I limiti metodologici

Caratteristiche dello studio		No.	%
Design	prospettico	108	86.4
	retrospettivo	9	7.2
	entrambi	8	6.4
Reclutamento	consecutivo	43	34.4
	mirato	24	19.2
	entrambi	5	4.0
	random	2	1.6
	non riportato	51	40.8
Cieco	doppio	8	6.4
	singolo (DAT)	7	5.6
	singolo (GS)	5	4.0
	non in cieco	2	1.6
	non riportato	103	82.4
Campioni	respiratori	95	76.0
	escreato	30	24.0
Gold standard	cultura	105	84.0
	clinica	3	2.4
	entrambi	17	13.6
Dati discrepanti	risolti	37	29.6
	non risolti	88	70.4

Ling DI et al., PloS ONE, February 2008, Issue 2. e1536

Parametri metodologici

- Studio prospettico eseguito in cieco
- Golden standard microbiologico e clinico
- Esclusione dei campioni raccolti da pazienti in terapia
- Valutazione separata dei campioni respiratori ed extrapolmonari
- Valutazione di 3 campioni a pz. per minimizzare la "uneven distribution" dei micobatteri nel campione
- Sensibilità e specificità calcolate per pz., non esclusivamente per campione

JCM Accepts, published online ahead of print on 22 December 2010
J. Clin. Microbiol. doi:10.1128/JCM.01839-10

Evaluation of the COBAS TaqMan MTB Test for Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

Yuan-Chieh Yang[1], Po-Liang Lu[1,2], Su Chiao Huang,[1] Yi-Shan Jenh[3], Ruwen Jou[4], Tsung Chain Chang[3]

TABLE 1. Comparison of the results of the COBAS TaqMan MTB Test with culture

Specimens	COBAS TaqMan MTB Test[a]	No. of specimens with culture result		Performance of the COBAS TaqMan MTB Test			
		Positive	Negative	% Sensitivity (95% CI)[d]	% Specificity (95% CI)	% PPV[b] (95% CI)	% NPV[c] (95% CI)
Smear positive (n = 118)	Positive	94	0	96.9 (93.5-1.0)	100 (1.0-1.0)	100 (1.0-1.0)	87.5 (74.3-1.0)
	Negative	3	21				
Smear negative (n = 975)	Positive	35	12	79.5 (67.6-91.5)	98.7 (98.0-99.4)	74.5 (62.0-86.9)	99.0 (98.4-99.7)
	Negative	9	919				
All (n = 1093)	Positive	129	12	91.5 (86.9-96.1)	98.7 (98.0-99.4)	91.5 (86.9-96.1)	98.7 (98.0-99.4)
	Negative	12	940				

[a]The results were adjusted after discrepant analysis.

[b]PPV, positive predictive value.

[c]NPV, negative predictive value.

[d]CI, confidence interval.

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2011, p. 173–176 Vol. 49, No. 1
0095-1137/11/$12.00 doi:10.1128/JCM.00694-10

Evaluation of Cobas TaqMan MTB PCR for Detection of *Mycobacterium tuberculosis*▽

Jeong Hyun Kim,[1]† Young Jae Kim,[1]† Chang-Seok Ki,[2]* Ji-Youn Kim,[3] and Nam Yong Lee[2]

Department of Laboratory Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, South Korea[1]; Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea[2]; and Center for Clinical Medicine, Samsung Biomedical Research Institute, Samsung Medical Center, Seoul, South Korea[3]

A total of 406 specimens collected from 247 patients

TABLE 1. Performance of the Cobas TaqMan and Amplicor PCRs based on culture results

Specimens	Test	Culture-positive (*n*)		Culture-negative (*n*)		Sensitivity/specificity (%)	PPV/NPV[a] (%)
		PCR$^+$	PCR$^-$	PCR$^+$	PCR$^-$		
All	TaqMan	19	5	7	375	79.1/98.2	73.1/98.7
	Amplicor	14	10	2	380	58.3/99.5	87.5/97.4
Respiratory	TaqMan	19	5	3	69	79.1/95.8	86.4/93.2
	Amplicor	14	10	1	71	58.3/98.6	93.3/87.6

[a] PPV, positive predictive value; NPV, negative predictive value.

Impatto Clinico dei DAT

Sospetto clinico	Microscopia	Diagnosi di TB	Utilità dei DATs
Alto	Positiva	Agevole	Limitata
Alto	Negativa	Presuntiva	Alta
Basso	Positiva	Incerta	Alta
Basso	Negativa	Improbabile	Bassa

The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis: results of a multicenter prospective trial.

Catanzaro A, et al. *JAMA* 2000; 283: 639-645

Impatto Clinico dei DAT

Table 3. Numbers Used for Calculating Sensitivity, Specificity, Positive and Negative Predictive Values by Level of Clinical Suspicion for Tuberculosis (TB)*

	Clinical Suspicion Level							
	Low (n = 224)		Intermediate (n = 68)		High (n = 46)		Total (N = 338)	
	TB Present	TB Absent	TB Present	TB Absent	TB Present	TB Absent	TB Present	TB Absent
Acid-fast bacilli smear†	5	9	5	11	33	2	43	22
Enhanced *Mycobacterium tuberculosis* Direct test†	10	7	15	0	35	0	60	7
Disease frequency (%)‡	12 (5)	NA	20 (29)	NA	40 (87)	NA	72 (21)	NA

*NA indicates not applicable.
†No. of positive results; defined as at least 1 positive test in a series of 1 to 6 per patient (median, 2.5).
‡Based on comprehensive clinical diagnosis as determined by expert panel review at end-of-study (3-mo follow-up) report.

Critical Use of Nucleic Acid Amplification Techniques To Test for *Mycobacterium tuberculosis* in Respiratory Tract Samples

Van den Wijngaert S, et al. J Clin Microbiol 2004; 42: 837-838

SELEZIONE DEI PAZIENTI

TABLE 2. Comparison of smear, culture, and NAT results for the three groups of patients analyzed

*: Cobas Amplicor Roche

Clinical suspicion	Patient group[d]	% Positive (no. of positive sample results/total no. tested) by:		
		M. tuberculosis culture[a]	NAT *	NTM culture[a,b]
High	1	89 (41/46)	89 (41/46)	2 (1/46)
Medium	2	20 (32/159)	16 (26/159)	0.6 (1/159)
Low	3	0 (0/56)	3.6 (2/56)[c]	0 (0/56)

[a] Culture, Löwenstein-Jensen–Mycobacteria Growth Indicator Tube method.
[b] NTM, non-tuberculosis mycobacterium.
[c] False positive results compared to *M. tuberculosis* culture results (the "gold standard").
[d] The results of smear tests for the members of group 1 were positive; those for the members of groups 2 and 3 were negative.

Costo-efficacia

- I test diagnostici a costo mediamente elevato possono ridurre la spesa sanitaria se:
  - Aumentano la accuratezza diagnostica
  - Riducono i tempi di degenza

Costo-efficacia dei DAT

Dipende da:

- No./anno di campioni microscopico-positivi
- Prevalenza di TB nei campioni microscopico-positivi
- Costo giornaliero dell'isolamento respiratorio

Si incrementa in caso di:

- Alta prevalenza di MNT
- Esecuzione dei DAT presso laboratori regionali di riferimento

Come implementare gli attuali sistemi
Problemi sul tappeto

Falsi negativi

- Diluizione del pellet
- Presenza di inibitori
  - Controllo interno di amplificazione (CIA)
- **Insufficiente estrazione del target**

Falsi positivi

- Contaminazioni crociate
- **Selezione dei pazienti**
- **Qual'è il gold standard?**

Sistemi commerciali di estrazione

Espy MJ, et al. Clin. Microbiol. Rev. 2006; 19: 165-256

Kit	Produttore	Principio	Formato
High pure	Roche	Fibre di vetro	Manuale
QIAmp	Qiagen	Gel di silice	Manuale
IsoQuick	Orca	Etanolo/tampone	Manuale
MagNA Pure	Roche	Silice magnetica	Automatizzato
BioRobot	Qiagen	Silice magnetica	Automatizzato
ABI Prism	Applied	Fibre di silicio	Automatizzato
NucliSens	bioMérieux	Silice magnetica	Automatizzato

Modifiche dei kit commerciali

DAT	Sample type	Analytical phases			
		Pre-testing	Testing	Post-testing	Outcome
Amplicor	Respir.	sample select.			> specificity
Amplicor	Extrapul.		DNA extraction		> sensitivity < specificity
E-MTD	All	sample select.	IAC		> sensitivity
E-MTD	Respir.			cut off values	> specificity
E-MTD	Respir.			cut off values	> specificity
E-MTD	CSF		RNA extraction		> sensitivity < specificity
ProbeTec	CSF		lysis	cut off values	> sensitivity

IDENTIFICAZIONE DA COLTURA

Genere Mycobacterium:
la scoperta di nuove specie

1980 → **56 specie**

Metodi tradizionali:

- Test biochimici
- Test colturali
- HPLC

2011 → **≅ 150 specie**

Metodi molecolari:

- DNA probe
- Sequenziamento

Burden of Unidentifiable Mycobacteria in a Reference Laboratory

ENRICO TORTOLI,[1]* ALESSANDRO BARTOLONI,[2] ERIK C. BÖTTGER,[3] STEFAN EMLER,[4] CARLO GARZELLI,[5] ENRICO MAGLIANO,[6] ANTONIA MANTELLA,[2] NALIN RASTOGI,[7] LAURA RINDI,[5] CLAUDIO SCARPARO,[8] AND PASQUALE URBANO[9]

J Clin Microbiol. 2001 Nov;39(11):4058-65

TABLE 2. Source and phenotypical and genotypical information about 72 unidentified mycobacteria in this study

Strain(s)	Origin[a]	Isolation (yr)	Source	Growth rate[b]	Pig-mentation[c]	Growth temp (°C)	Closest identification by:		
							Conventional tests[d]	HPLC[d]	Genetic sequencing[e]
FI-12100 and FI-25197	Vicenza-Milan	1995–2000	Sputum, blood from AIDS patient	S	S/N	25–37	*M. lentiflavum*	MAC	*Mycobacterium* sp. strain MCRO33 (7)*
FI-22895	FI	1997	Sputum	S	S	25–37	*M. interjectum*	MAC	*M. paraffinicum*
FI-24599	Vicenza	1999	Venous central catheter tip	R	S	25–37	*M. neoaurum*	*M. neoaurum*	*Mycobacterium* sp. strain IWGMT90161 (14)/*M. simiae*
FI-2389 and 2 others	Milan 2 + FI	1996–1998	Urine, gastric juice, sputum	R	S/N	25–37	Runyon group iv	*M. neoaurum*	*Mycobacterium* sp. strain SRB1151-113[f]
FI-1191	FI	1999	Urine	R	S	25–37	*M. vaccae*	*M. fallax*	*M. neoaurum*/*M. fallax*
FI-27599	Vicenza	1999	Bronchial aspirate	S	S	25–37	*M. gordonae*	*M. diernhoferi*	*M. fortuitum*/"*M. ratisbonense*"[g]
FI-7494	Milan	1996	Sputum	R	S	25–37	Runyon group iv	*M. fortuitum*	*M. aichuense*/*M. diernhoferi*
FI-11097	Milan	1997	Sputum	R	P	25–37	Runyon group iv	*M. fortuitum*	*M. peregrinum*/*M. gilvum*
FI-17697	Milan	1997	Sputum	R	S	25–37	*M. neoaurum*	*M. phlei*	*M. peregrinum*/*M. gilvum*
FI-16194	FI	1994	Bronchial aspirate	R	N	25–37	*M. mucogenicum*	*M. fallax*	"*M. ratisbonense*"[g]
FI-100	Vicenza	2000	Sputum	R	N	25–37	*M. mucogenicum*	MCLO	"*M. ratisbonense*"*[g]
FI-23299	Vicenza	1999	Water	R	N	25–45	*M. fallax*	*M. gordonae*	*M. fortuitum*
FI-29396	Vicenza	1996	Sputum	R	S	25–37	*M. vaccae*	*M. duvalii*	*M. gilvum*
FI-26200	Vicenza	2000	Bronchial aspirate	R	S	25–37	*M. gordonae*	MCLO	*M. aurum*
FI-19499	Vicenza	1999	Bronchial aspirate	R	N	25–37	*M. mucogenicum*	*M. aurum*	Mycobacterium MO183[g]/ "*M. ratisbonense*"[g]

DNA-*probe* commerciali

- **AccuProbe** (GenProbe)
- **INNO LiPA *Mycobacterium*** (Innogenetic)
- **GenoType Mycobacteria** (Hain)

- Enorme risparmio di tempo rispetto all'identificazione tradizionale
- Specificità elevata
  - ✓Disponibili per un numero limitato di specie
  - ✓Costose

AccuProbe (Gen Probe)

- Bersaglio: Sequenza del 16S rRNA
- Reazione: ibridizzazione in fase liquida, direttamente dalle colonie - Massa dipendente
- Rivelazione: in chemioluminescenza (*HPA*)
- Specie identificate:
  - ***M. tuberculosis* complex**
  - ***M. kansasii***
  - ***M. gordonae***
  - ***M. avium* complex** o separatamente ***M. intracellulare*** e ***M. avium***
- Limiti:
  - ✓ test a cascata
  - ✓ non disponibili per specie comuni in Europa ma rare negli USA

AccuProbe: le reazioni crociate

- *M. avium*
- *M. intracellulare*
  - *M. arosiense, M. chimaera, M. nebraskense, M. saskatchewanense*
- *M. avium* complex
  - *M. arosiense, M. chimaera, M. colombiense, M. nebraskense, M. palustre, M. paraffinicum, M. saskatchewanense, M. vulneris*
- *M. tuberculosis* complex
  - *M. holsaticum*
- *M. kansasii*
- *M. gordonae*

INNO LiPA Mycobacteria (Innogenetics)

- Bersaglio: Regione spaziatrice presente tra il gene 16S e 23S del rRNA (ITS = internal transcribed spacer)

- Reazione: ibridizzazione inversa, con 22 sonde immobilizzate su una striscia di cellulosa, del bersaglio amplificato mediante *PCR* (*primer* biotinilati)

- Rivelazione: colorimetrica (avidina-perossidasi)

- Limiti: alcune reattività crociate con specie rare

Marker line

1 – Conjugate control
2 – Myc genus
3 – MTB complex
4 – M. kansasii I
5 – M. kansasii II
6 – M. kansasii III, IV, V
7 – M. xenopi
8 – M. gordonae
9 – M. genavense
10 – M. simiae
11 – M. marinum + M. ulcerans
12 – M. celatum
13 – MAIS complex
14 – M. avium
15 – M. intracelulare 1
16 – M. chimaera
17 – M. scrofulaceum
18 – M. malmoense
19 – M. haemophilum
20 – M. chelonae I, II, III, IV
21 – M. chelonae III
22 – M. chelonae I
23 – M. fortuitum complex
24 – M. smegmatis

INNO LiPA Mycobacteria V.2: Specificità delle sonde

- di genere: genere *Mycobacterium*
- di specie: (*M. tuberculosis* complex, *M. xenopi*, *M. gordonae*, *M. genavense*, *M. simiae*, *M. marinum/ulcerans*, *M. celatum*, *M. avium*, *M. intracellulare*, *M. chimaera*, *M. scrofulaceum*, *M. malmoense*, *M. haemophilum*, *M. fortuitum-peregrinum* complex, *M. smegmatis*)
- intraspecifica: *M. kansasii* (tipi I, II, III, IV,V, *M. gastri*) e *M. chelonae* (gruppi I, II, III, IV)
- multipla: MAIS

Evaluation of INNO-LiPA MYCOBACTERIA v2: improved reverse hybridization multiple DNA probe assay for mycobacterial identification.

Tortoli E, et al. J Clin Microbiol. 2003 Sep;41(9):4418-20

Sensitivity and specificity of INNO-LiPA MYCOBACTERIA v2

Assay	% Sensitivity	% Specificity
Genus-specific probe	100	100
Species -specific probe	92.2	100

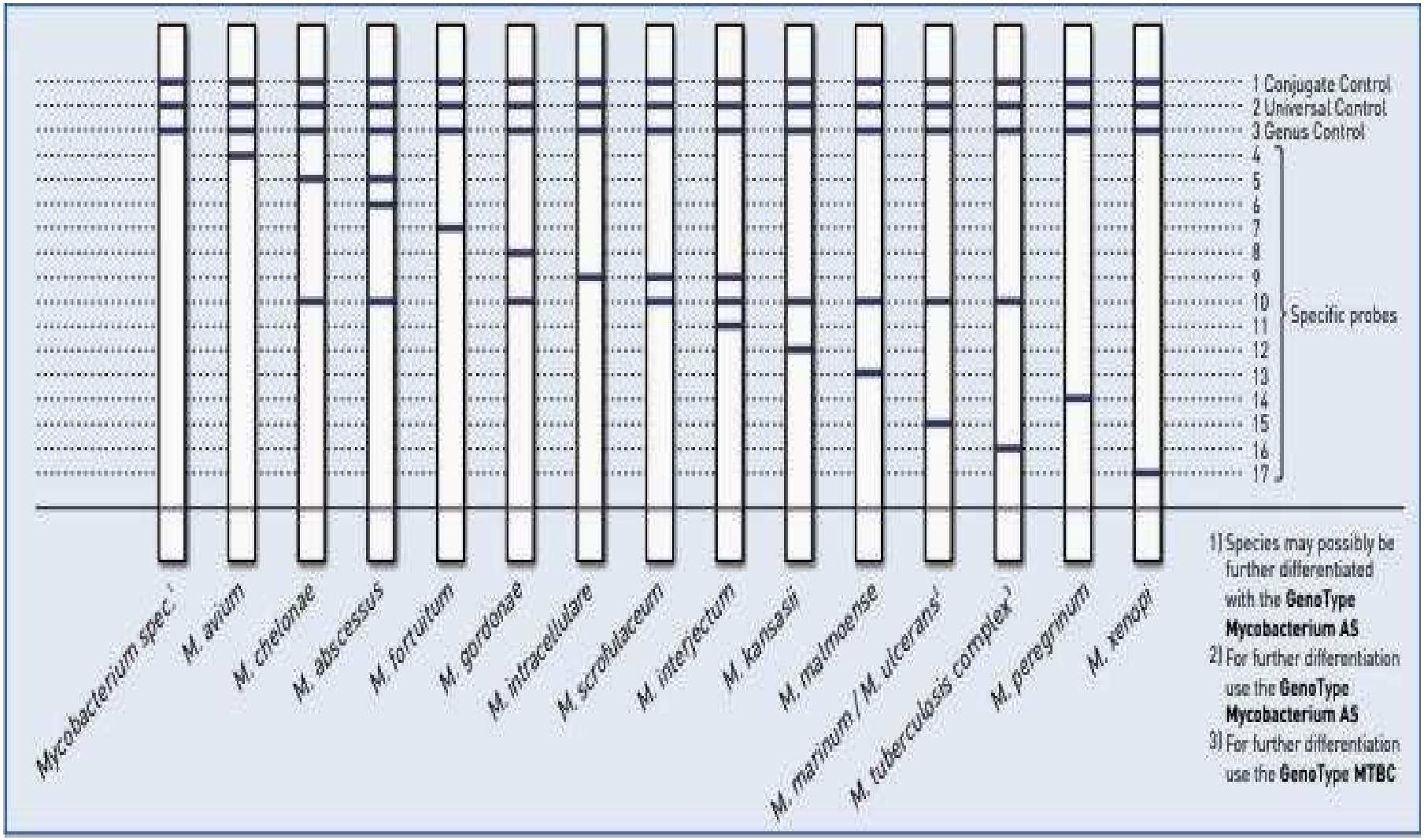
INNO LiPA: le reazioni crociate

- *M. tuberculosis* complex
- *M. kansasii* (differenziazione dei tipi I, II, III-IV-V-*M. gastri*)
- *M. xenopi*
- *M. gordonae*
- *M. genavense*
- *M. simiae*
  - *M. sherrisii*
- *M. marinum-M. ulcerans*
  - *M. pseudoshottsii, M. shottsii*
- *M. celatum*
- MAC-*M. scrofulaceum-M. malmoense-M. hemophilum*
  - *M. arosiense, M. heidelbergense, M. mantenii, M. nebraskense, M. paraffinicum, M. parascrofulaceum*
- *M. intracellulare*
- *M. chimaera*
  - *M. nebraskense, M. paraffinicum*
- *M. scrofulaceum*
  - *M. parascrofulaceum*
- *M. malmoense*
- *M. haemophilum*
- *M. chelonae-M. abscessus* (differenziazione dei tipi I, III, II-IV)
- *M. fortuitum- M. peregrinum*
  - *M. alvei, M. conceptionense, M. goodii, M. mageritense, M. neworleansense, M. senegalense, M. septicum, M. thermoresistibile, M. wolinskyi*
- *M. smegmatis*

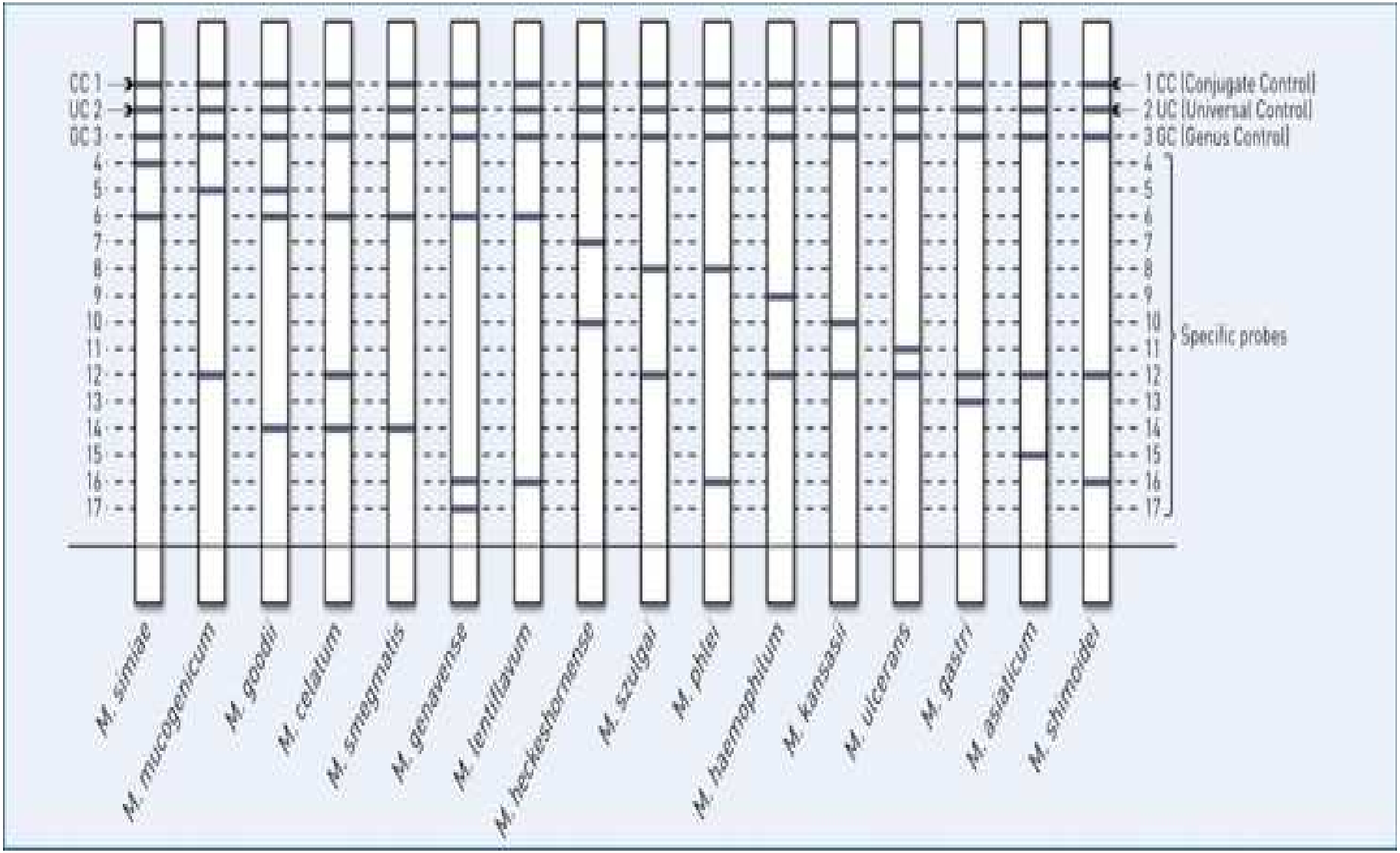
GenoType Mycobacterium (Hain)

- Bersaglio: 23S rDNA
- Reazione: ibridizzazione inversa del bersaglio amplificato mediante *PCR* (*primer* biotinilati) con varie sonde immobilizzate su una striscia di cellulosa
- Rivelazione: colorimetrica (avidina-perossidasi)
- Limiti:
  - ✓ incorretta identificazione dei MAC non-*M. avium*, non-*M. intracellulare*
  - ✓ alcune reattività crociate con specie rare
  - ✓ numerose identificazioni non univoche
- Presenza di due diversi kit di rivelazione
  - GenoType Mycobacterium CM (Common Mycobacteria)
  - GenoType Mycobacterium AS (Additional Species)

Mycobacterium CM



Mycobacterium AS



Evaluation of the New GenoType Mycobacterium Assay for Identification of Mycobacterial Species

Russo C, et al. J Clin Microbiol. 2006 Feb;44(2):334-9.

TABLE 2. Sensitivity and specificity of GenoType assay

Assay	% Sensitivity	% Specificity
Genus-specific probe		
CM strip	98.9	88.9
AS strip	99.4	100
Species-specific probes		
CM strip	97.9	92.4
AS strip	99.3	99.4

GenoType: le reazioni crociate

- *Gram +*
- *Mycobacterium* genere
- *M. avium*
- *M. chelonae-M. immunogenum*
- *M. abscessus-M. immunogenum*
- *M. fortuitum*
  - *M. boenickei,M. farcinogenes, M. houstonense, M. neworleansense, M. parafortuitum, M. porcinum, M. senegalense, M. setense,*
- *M. fortuitum-M. mageritense*
  - *M. conceptionense, M. wolinskyi*
- *M. gordonae*
- *M. intracellulare*
  - *M. arosiense, M. chimaera, M. colombiense, M. mantenii, M. saskatchewanense,* MAC
- *M. scrofulaceum-M. parascrofulaceum-M. paraffinicum*
  - *M. alsiense*
- *M. interjectum*
- *M. kansasii*
  - *M. gastri*
- *M. malmoense-M. haemophilum-M.palustre-M. nebraskense*
- *M. marinum-M. ulcerans*
- *M. tuberculosis* complex
  - *M. ryiadhense*
- *M. peregrinum-M. alvei-M. septicum*
- *M. xenopi*

- *Mycobacterium* genere
- *M. simiae*
  - *M. sherrisii*
- *M. mucogenicum*
  - *M. aubagnense, M. llatzerense, M. phocaicum*
- *M. goodii*
- *M. celatum* I, III
- *M. smegmatis*
- *M. genavense-M. triplex*
- *M. lentiflavum*
- *M. heckeshornense*
- *M. szulgai-M. intermedium*
- *M. phlei*
- *M. haemophilum-M. nebraskense*
- *M. kansasii*
- *M. ulcerans*
- *M. gastri*
- *M. asiaticum*
- *M. shimoidei*

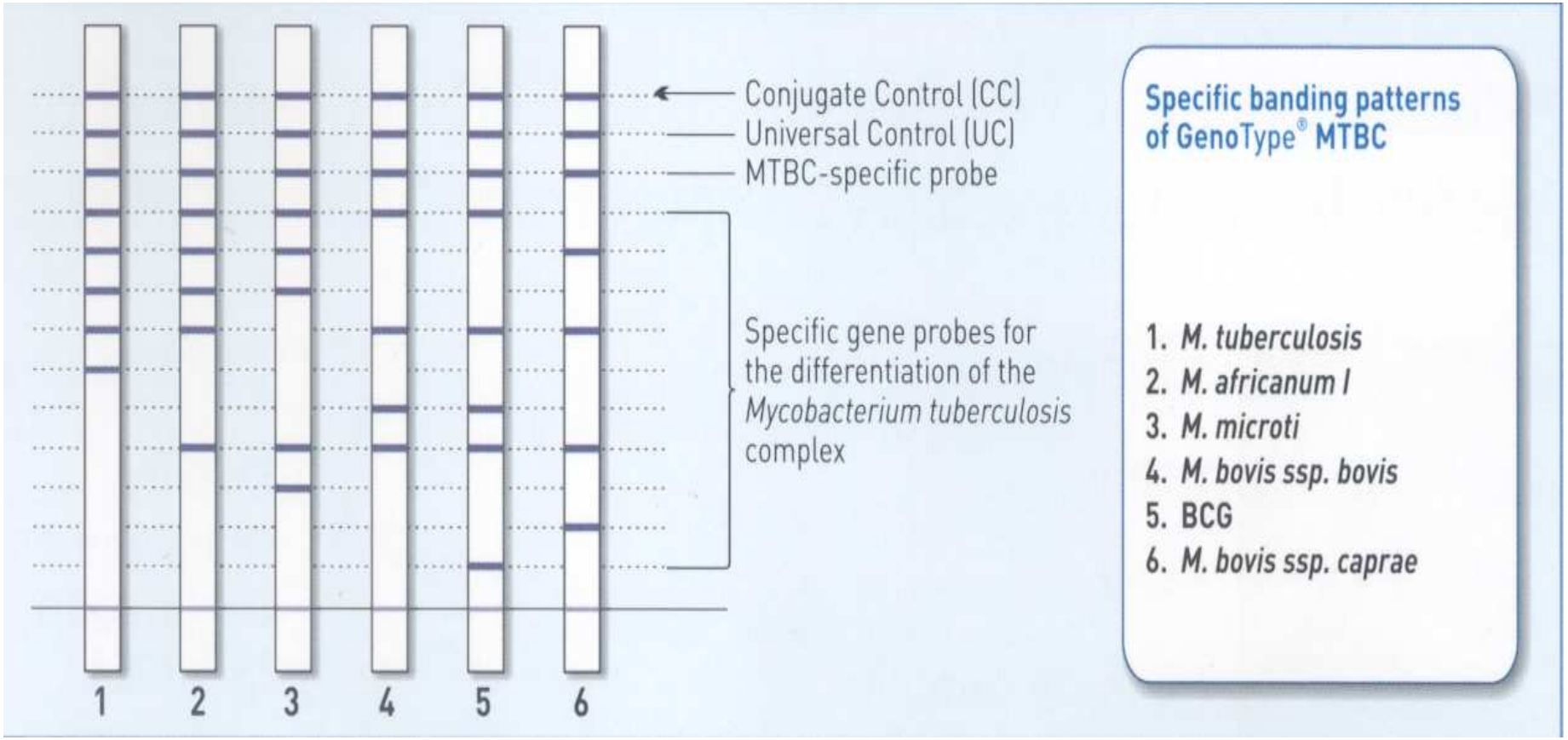
GenoType MTBC (Hain)

- Bersagli:
  - ✓ gene 23S rDNA - gene *gyrB* - regione RD1
- Reazione: ibridizzazione inversa, con 9 sonde immobilizzate su una striscia di cellulosa, del bersaglio amplificato mediante *PCR* (*primer* biotinilati)
- Rivelazione: colorimetrica (avidina-perossidasi)
- Da colture in terreno solido e terreno liquido

MTB complex

- ✓ *M. tuberculosis complex*
- ✓ *M. tuberculosis*
- ✓ *M. bovis*
- ✓ *M. bovis* BCG
- ✓ *M. caprae*
- ✓ *M. africanum sottotipo I*
- ✓ *M. microti*

GenoType® MTBC



Limiti: non riconoscimento di *M. africanum* tipo II, di *M. canettii* e di *M. pinnipedii*

Direct Comparison of the GenoType MTBC and Genomic Deletion Assays in Terms of Ability To Distinguish between Members of the *Mycobacterium tuberculosis* Complex in Clinical Isolates and in Clinical Specimens

Somoskovi A, et al. J Clin Microbiol. 2008 May;46(5):1854-7

192 isolates and 79 smear-positive clinical specimens

TABLE 3. Results of GenoType MTBC assay used directly on 59 smear-positive and *M. tuberculosis* complex-positive clinical specimens

PCR-based deletion analysis identification for growth-positive cultures (reference method) (*n*)	No. (%) of conclusive GenoType MTBC assay-positive results with smear-positive and *M. tuberculosis* complex-positive clinical specimens
M. tuberculosis (48)	44[a] (91.6)
M. africanum (6)	6 (100)
M. bovis (4)	4 (100)
M. bovis BCG (1)	1 (100)
Total (59)	55 (93.2)

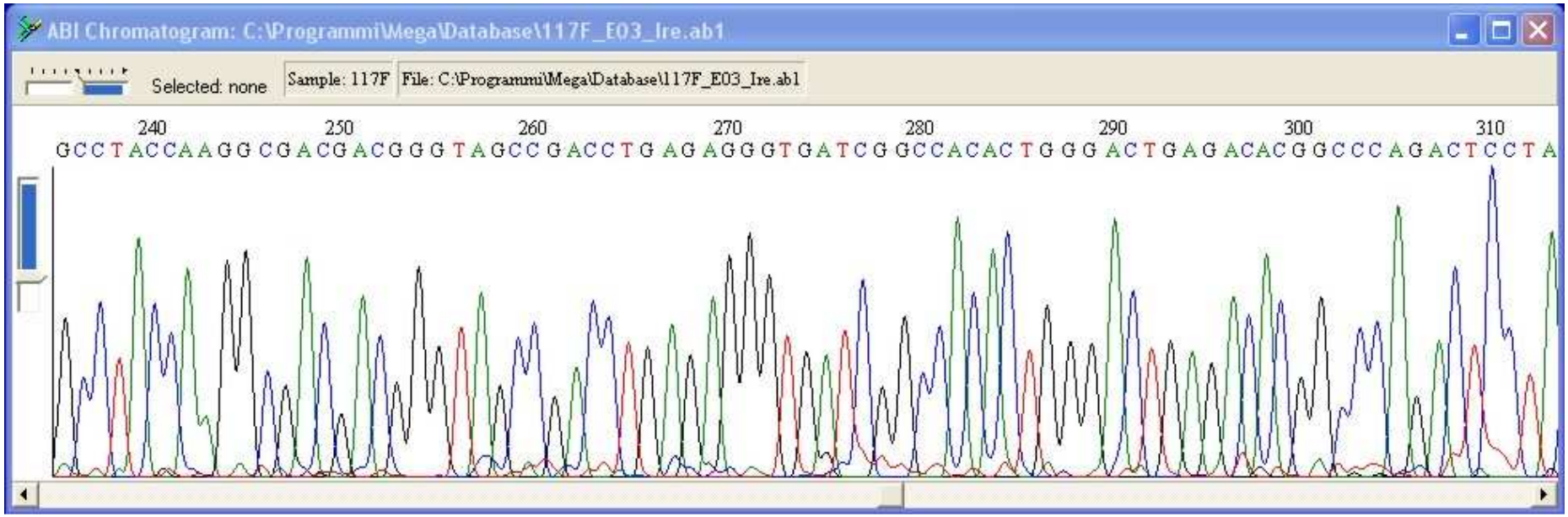
[a] Amplification was unsuccessful for three genes (23S rRNA, RD1, and *gyrB*) in four specimens with the GenoType MTBC assay; therefore, a conclusive result could not be obtained.

Il sequenziamento in microbiologia

- Identificazione
  - Sequenziamento di regioni specie-specifiche
- Antibiogramma
  - Sequenziamento di regioni in cui possono aversi mutazioni associate alla resistenza ai farmaci
- Filogenesi
  - Confronto della sequenza della medesima regione in specie diverse, per ricostruirne la storia evolutiva

Identificazione mediante sequenziamento

- Scelta della regione da sequenziare
  - 16S rDNA
  - *Internal transcribed spacer*
  - 23S rDNA
  - *hsp65*
  - *rpoB*
- Sequenziamento
- Confronto della sequenza con quelle presenti in un database



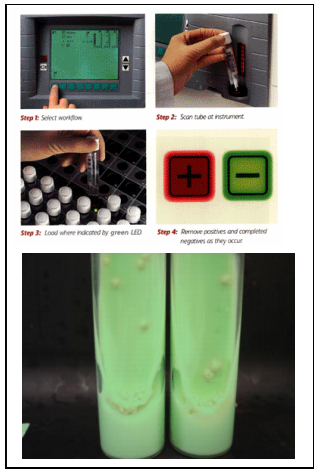
Le varie possibilità

- Identità 100% con una specie nota (ceppo di riferimento)
- Identità 100% con un ceppo (non di riferimento) appartenente ad una specie nota
- Identità 100% con una sequenza non appartenente a specie conosciute
- Identità <100%
  - Verifica delle discordanze ed eventuale correzione della sequenza e, se confermate:
    - Nuovo *sequevar*
    - Nuova specie

- Identificazione mediante Sequenziamento:
  - Metodo di riferimento
  - Sistema aperto
- Problematiche legate ai database
- La scelta fra le identificazioni proposte

Susceptibility Testing in *M. tuberculosis*

Metodi Fenotipici (valutazione dela crescita in terreno solido/liquido in presenza del farmaco):



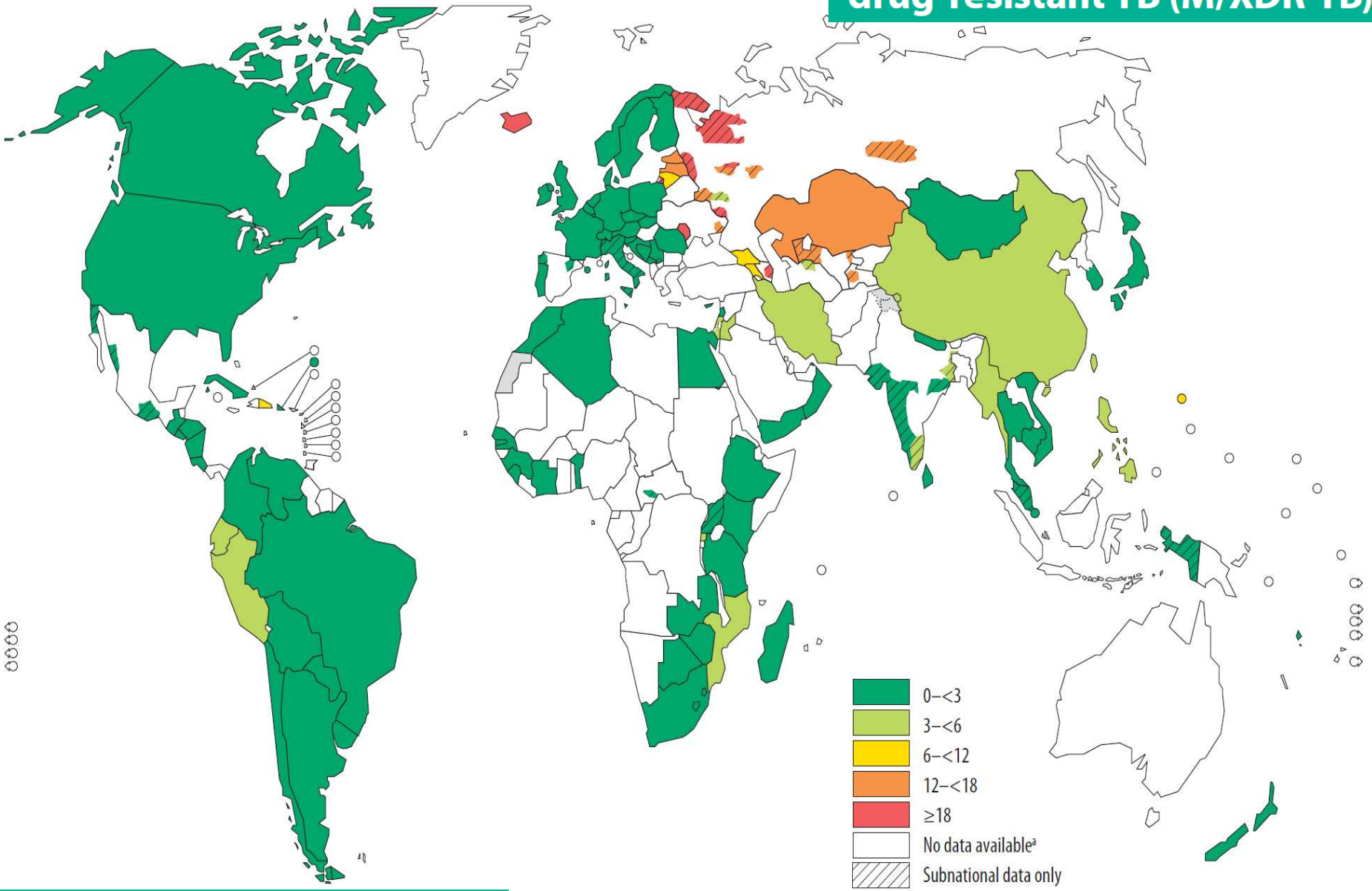
- Costo-efficace
- Semplice da eseguire più complessa da standardizzare
- Risultati disponibili in settimane/mesi

Metodi Moleculari (identificazione delle mutazioni responsabili di resistenza):

- (generalmente) costosi
- Difficoltà di esecuzione, limitato ad alcuni targets
- Risultati disponibili in ore
- Non richiede ceppo vitale, indipendente da inquinamento del campione

Multidrug and extensively drug-resistant TB (M/XDR-TB)

MAP 3 Distribution of proportion of MDR-TB among new TB cases, 1994–2009



[a] Australia, Democratic Republic of the Congo, Fiji, Guam, New Caledonia, Solomon Islands and Qatar reported data on combined new and previously treated cases.

XDR-TB

Countries reporting XDR-TB as of March 2010

XDR-TB Findings:

- 58 countries reported at least one case of XDR-TB as of March 2010
- Representative data from 46 countries
- 5.4% of MDR-TB cases have XDR-TB

There are thought to be 25,000 cases of XDR-TB emerging every year

APPROCCIO CHEMIOTERAPICO ALLA TUBERCOLOSI

Diverso dalle altre malattie batteriche per:

- Lungo tempo di replicazione dei micobatteri
- Fase di quiescenza
- Crescita in situazioni metaboliche molto diverse
- Crescita in ambienti molto diversi (presenza di ossigeno, microarofilia, basso pH)
- Necessità di più farmaci attivi contemporaneamente

Resistance in *M. tuberculosis*

Due exclusively to chromosomal mutations

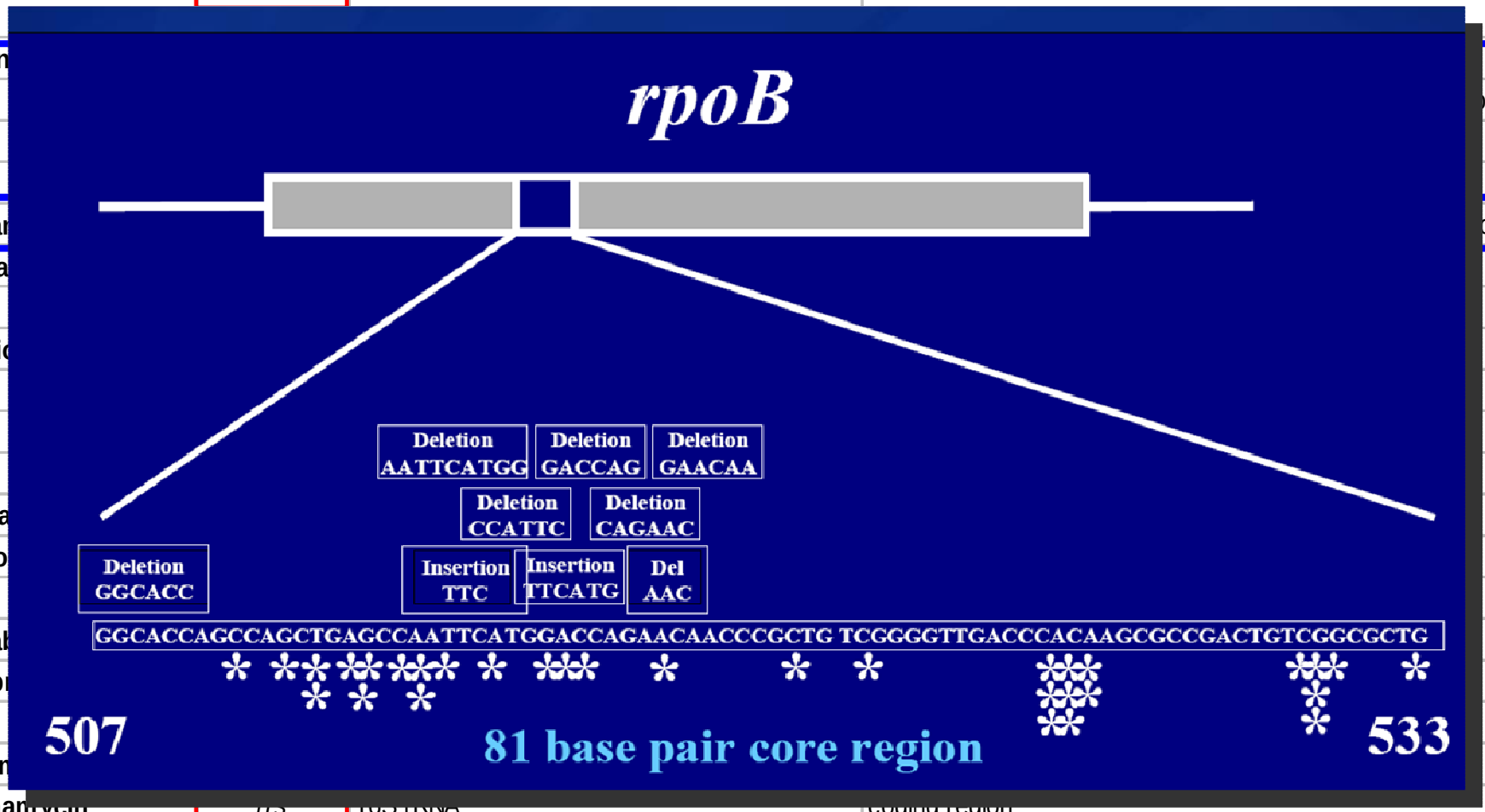
- Mutations responsible of drug resistance occur spontaneously with variable frequencies ($1/10^6$-$1/10^8$)
- Resistance is the results of the selection of resistant mutants due to inadequate therapy

The use of at least two active drugs decreases the occurrence of resistances

- **DST must be: reliable and rapid to perform**

Geni coinvolti nella resistanza ai maggiori farmaci anti-tubercolari

Drug	Gene	Gene product	Mutations
Streptomycin	*rpsL*	12S ribosomal protein	Coding region (60%)
Ison			
Rifa			
Etha			
Ethi			
Pyra			
Fluo			
Rifal			
Capr			
Vior			
Kanamycin	*rrs*	16S rRNA	coding region
Amikacin	*rrs*	16S rRNA	coding region
D-Cycloserine	*alr*	D-Ala racemase	promoter region
Para-salicylic acid	*thyA*	Thymidylate synthase	coding region



Resistenza alla Rifampicina: il "gold target"

- Farmaco chiave nel regime anti TB
- Bassa frequenza di mutazioni spontanee
- Mutazioni concentrate in una regione hot-spot in *rpoB* gene

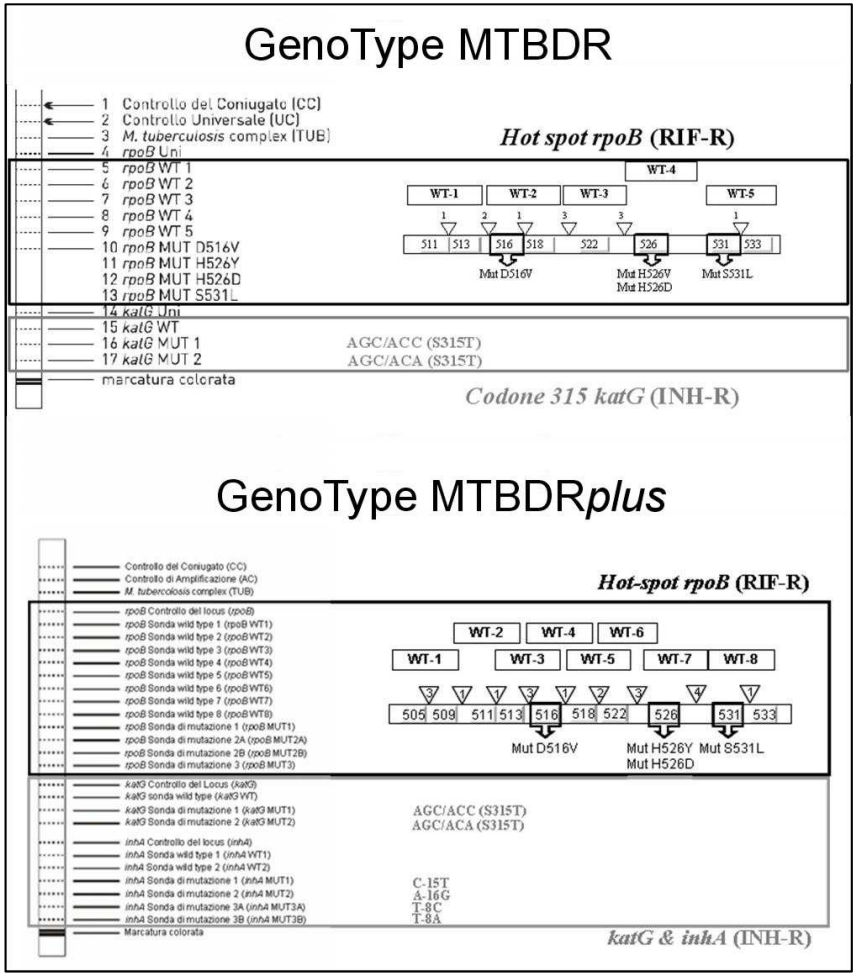
Ottimo candidato per la diagnosi molecolare di resistenza

Identificazione delle mutazioni che conferiscono resistenza a Isoniazide: "i problemi"

- Mutazioni in più geni strutturali e regolatori (*inhA*) o mutazioni multiple nello stesso gene (*katG, ahpC*)
- Solo alcune mut (*katG*) correlanano con il fenotipo di resistenza ad alta concentrazione, altre non hanno significato clinico (?)
- Frequenza di mutazione diversa su base geografica
- Relazione tra over-expressione di *ahpC* e fenotipo resistente non chiara

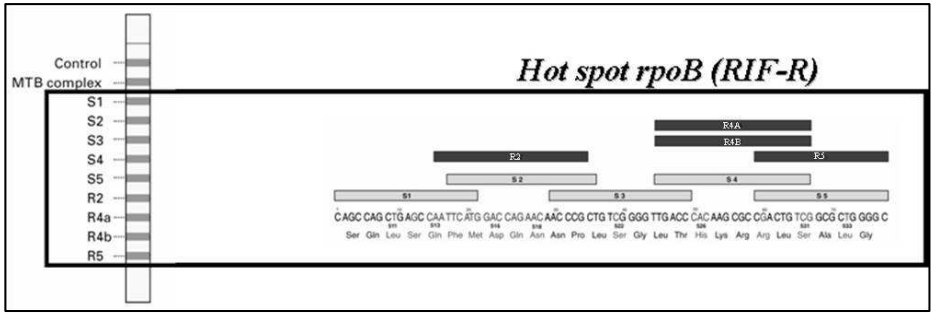
Test commerciali per la farmacoresistenza

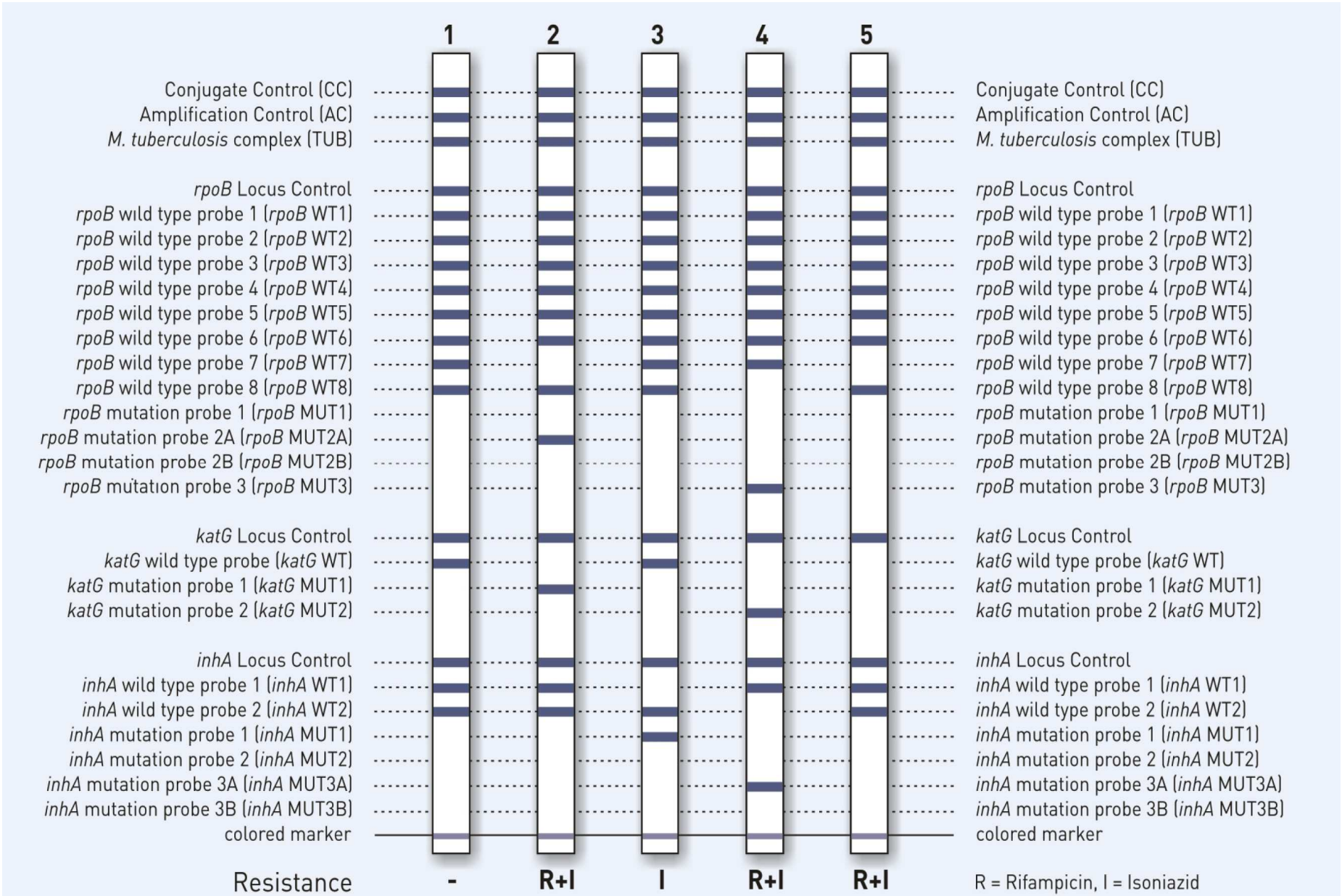
Hain Lifescience



Innogenetics

INNO-LiPA-Rif.TB





Reaction zones of GenoType® MTBDRplus (examples)

A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis

Morgan M, et al. BMC Infect Dis 2005, 5:62

Pooled Sensitivity = 0.97 (0.95 to 0.98)
Pooled Specificity = 0.99 (0.98 to 1.00)

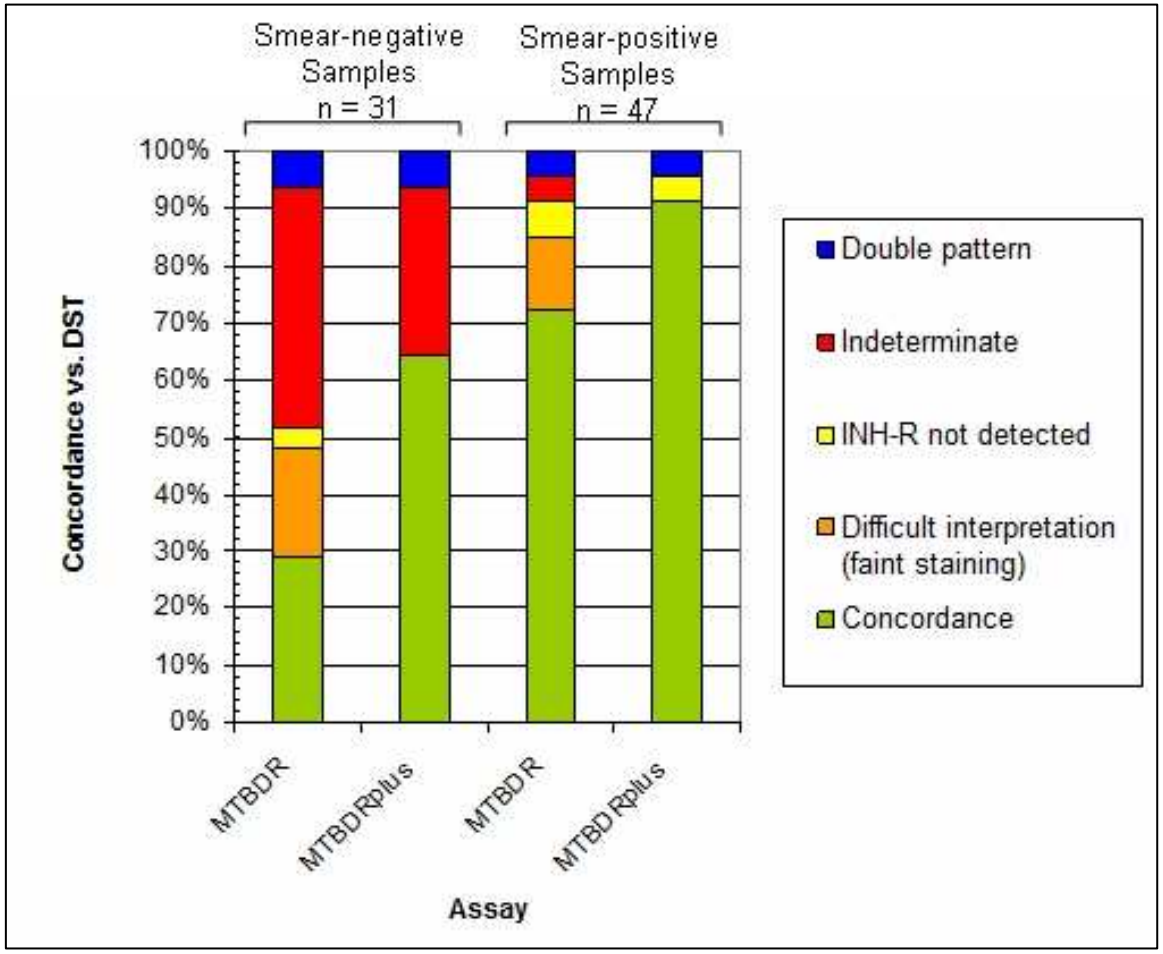
INNO-LIPA Rif. TB

Table 1: Description of studies included in meta-analysis.

Author (year)	Country	Reference Test	Blinded to reference test?	Sample	Sample size (# resistant / # sensitive)	Sensitivity (95% CI)	Specificity (95% CI)
Ahmad (2002)	Kuwait	BACTEC 460	Not Specified	Isolate	29/12	0.97 (.82–1.0)	1.0 (.74–1.0)
De Oliveira (1998)	Brazil	Proportion	Not Specified	Isolate	113/15	0.97 (.92–.99)	1.0 (.78–1.0)
Gamboa (1998)	Spain	BACTEC 460	Not Specified	Isolate	46/13	1.0 (.92–1.0)	1.0 (.75–1.0)
Hirano (1999)	Japan	Proportion	Not Specified	Isolate	90/26	0.92 (.85–.97)	1.0 (.87–1.0)
Johansen (2003)	Denmark	BACTEC 460	Not Specified	Isolate	35/24	0.97 (.85–1.0)	1.0 (.86–1.0)
Jureen (2004)	Sweden	BACTEC 460	Not Specified	Isolate	27/26	1.0 (.87–1.0)	0.92 (.75–.99)
Lemus (2004)	Belgium	BACTEC 460, Proportion	Yes	Isolate	10/10	1.0 (.69–1.0)	1.0 (.69–1.0)
Rossau (1997)	Belgium	Proportion	Not Specified	Isolate	203/61	0.98 (.95–.1.0)	1.0 (.94–1.0)
Sintchenko (1999)	Australia	BACTEC 460	Not Specified	Isolate	22/11	0.96 (.77–1.0)	1.0 (.72–1.0)
Somoskovi (2003)	USA	Proportion	Not Specified	Isolate	64/37	0.95 (.87–.99)	1.0 (.91–1.0)
Srivastava (2004)	India	MIC	Not Specified	Isolate	45/10	0.82 (.68–.92)	1.0 (.69–1.0)
Tracevska (2002)	Latvia	BACTEC 460	Not Specified	Isolate	34/19	1.0 (.90–1.0)	1.0 (.82–1.0)
Traore (2000)	Belgium	Proportion	Not Specified	Isolate	266/145	0.99 (.96–1.0)	1.0 (.98–1.0)
Watterson (1998)	England	BACTEC 460, Proportion	Not Specified	Isolate	16/16	1.0 (.80–1.0)	0.94 (.70–1.0)
De Beenhouwer (1995)	Belgium	Proportion	Not Specified	Clinical Specimen	21/46	0.91 (.70–1.0)	1.0 (.92–1.0)
Gamboa (1998)	Spain	BACTEC 460	Not Specified	Clinical Specimen	46/13	0.98 (.89–1.0)	1.0 (.75–1.0)
Johansen (2003)	Denmark	BACTEC 460	Not Specified	Clinical Specimen	26/21	1.0 (.87–1.0)	1.0 (.84–1.0)
Watterson (1998)	England	BACTEC 460, proportion	Yes	Clinical Specimen	10/24	0.80 (.44–.98)	1.0 (.86–1.0)

Genotype MTBDR*plus*: a Further Step toward Rapid Identification of Drug-Resistant *Mycobacterium tuberculosis*

Miotto P, et al. *J Clin Microbiol.* 2008 Jan;46(1):393-4



New generation of LiPA performs better in both Smear+ and Smear- samples

GenoType MTBDR*plus* Assay for Molecular Detection of Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis* Strains and Clinical Samples

Lacoma A, et al. J Clin Microbiol. 2008 Nov;46(11):3660-7

62 clinical isolates and
65 clinical samples

TABLE 2. MTBDR*plus* assay results according to Bactec 460TB system results for the 62 clinical strains

MTBDR*plus* test result	Bactec 460TB system result (no. [%] of strains)			
	INH		RIF	
	Susceptible (n = 14)	Resistant (n = 48)	Susceptible (n = 50)	Resistant (n = 12)
Susceptible	14 (100)	13 (27)	50 (100)	1 (8.3)
Resistant	0	35 (73)	0	11 (91.7)

Sensitivity

Sensitivity

The NEW ENGLAND JOURNAL of MEDICINE

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.

Xpert MTB/Rif: FIND Evaluation studies

Rigorous performance evaluation at 5 sites (>1500 TB suspects)
Included 2 sites with high HIV prevalence (80%) & 2 with high MDR prevalence (>30%)

	UPCH
HIV	2%
TB (C+)	61%
MDR TB	7%

Peru
UPCH

Germany
NRL

Azerbaijan
STI

India
Hinduja

	STI
HIV	5%
TB (C+)	42%
MDR TB	31%

	Hinduja
HIV	5%
TB (C+)	60%
MDR TB	67%

South Africa
UCT
SAMRC

	UCT	SAMRC
HIV	77%	72%
TB (C+)	39%	13%
MDR TB	10%	9%

Table 2. Overall Sensitivity and Specificity of the MTB/RIF Test, According to the Number of Tests per Patient, as Compared with Three Smears and Four Cultures.*

Site and No. of Tests	Sensitivity			Specificity
	All Culture-Positive	Smear-Positive and Culture-Positive	Smear-Negative and Culture-Positive	No Tuberculosis
Site				
Lima, Peru				
Correct — no./total no. (%)	209/211 (99.1)	199/199 (100)	10/12 (83.3)	102/102 (100)
95% CI	96.6–99.7	98.1–100.0	55.2–95.3	96.4–100.0
Baku, Azerbaijan				
Correct — no./total no. (%)	144/149 (96.6)	80/80 (100.0)	64/69 (92.8)	68/70 (97.1)
95% CI	92.4–98.6	95.4–100.0	84.1–96.9	90.2–99.2
Cape Town, South Africa				
Correct — no./total no. (%)	142/148 (95.9)	95/96 (99.0)	47/52 (90.4)	186/189 (98.4)
95% CI	91.4–98.1	94.3–99.8	79.4–95.8	95.4–99.5
Durban, South Africa				
Correct — no./total no. (%)	43/45 (95.6)	30/30 (100.0)	13/15 (86.7)	213/219 (97.3)
95% CI	85.2–98.8	88.6–100.0	62.1–96.3	94.2–98.7
Mumbai, India				
Correct — no./total no. (%)	185/188 (98.4)	162/162 (100.0)	23/26 (88.5)	35/36 (97.2)
95% CI	95.4–99.5	99.7–100.0	71.0–96.0	85.8–99.5
No. of MTB/RIF tests				
3 Samples (2 pellet and 1 direct)				
Correct — no./total no. (%)	723/741 (97.6)	566/567 (99.8)	157/174 (90.2)	604/616 (98.1)
95% CI	96.2–98.5	99.0–100.0	84.9–93.8	96.6–98.9
2 Samples (1 pellet and 1 direct)				
Correct — no./total no. (%)†	1423/1482 (96.0)	1127/1134 (99.4)	296/348 (85.1)	1215/1232 (98.6)
95% CI	94.6–97.1	98.6–99.7	79.7–89.2	97.5–99.2
1 Sample (direct)				
Correct — no./total no. (%)	675/732 (92.2)	551/561 (98.2)	124/171 (72.5)	604/609 (99.2)
95% CI	90.0–93.9	96.8–99.0	65.4–78.7	98.1–99.6

Table 3. Sensitivity and Specificity of the MTB/RIF Test for the Detection of Rifampin and Multidrug Resistance, as Compared with Phenotypic Drug-Susceptibility Testing Alone and in Combination with Sequencing of Discrepant Cases, According to Site.*

Site and Total	Phenotypic Drug-Susceptibility Testing†		Phenotypic Drug-Susceptibility Testing and Discrepant Resolution by Sequencing†	
	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance
Lima, Peru — no./total no. (%)	16/16 (100.0)	190/193 (98.4)	19/19 (100.0)	190/190 (100.0)
Baku, Azerbaijan — no./total no. (%)	47/49 (95.9)	90/94 (95.7)	51/52 (98.1)	90/90 (100.0)
Cape Town, South Africa — no./total no. (%)	15/16 (93.8)	126/126 (100.0)	15/15 (100.0)	126/126 (100.0)
Durban, South Africa — no./total no. (%)	3/3 (100.0)	38/38 (100.0)	3/3 (100.0)	38/38 (100.0)
Mumbai, India — no./total no. (%)	119/121 (98.3)	61/64 (95.3)	121/122 (99.2)	62/62 (100.0)
Total for rifampin resistance				
Correct — no./total no. (%)	200/205 (97.6)	505/515 (98.1)	209/211 (99.1)	506/506 (100.0)
95% CI — %	94.4–99.0	96.5–98.9	96.6–99.7	99.2–100.0
Total for multidrug resistance				
Correct — no. /total no. (%)	195/200 (97.5)		197/199 (99.0)	
95% CI — %	94.3–98.9		96.4–99.7	

* Multidrug resistance is defined as resistance to both rifampin and isoniazid. Of 723 culture-positive samples, 720 were analyzed for rifampin resistance because results on the MTB/RIF test were indeterminate in 3 cases. During blinded sequencing of 15 discrepant samples, *rpoB* mutations were identified in 9 samples that were rifampin-sensitive on phenotypic drug-susceptibility testing. A wild-type allele was identified in 1 sample, which had been reported as resistant on phenotypic drug-susceptibility testing. Mixed infections were identified in 3 samples and were excluded from the analysis after discrepant resolution. In 2 samples, sequencing confirmed the phenotypic result: *rpoB* mutation 516 GTC was detected in 1, and 531 TTG in the other.

† This is the reference standard for the comparison with the MTB/RIF test.

Boheme at al, NEJM 2010

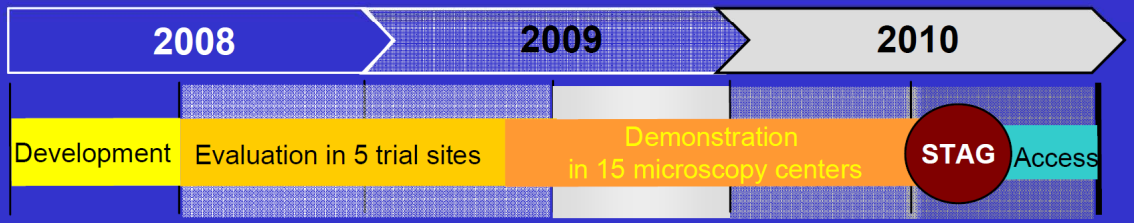
High tech in low tech settings: Xpert™ MTB for TB & RIF resistance detection

Cepheid

Automated Sample Prep,

Amplification and Detection

<120 minutes



Challenges downstream:
Meeting the target price

A technology platform:

- TB & Rif Resistance
- Potential for HIV viral load
- Potential for …

FIND
FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS

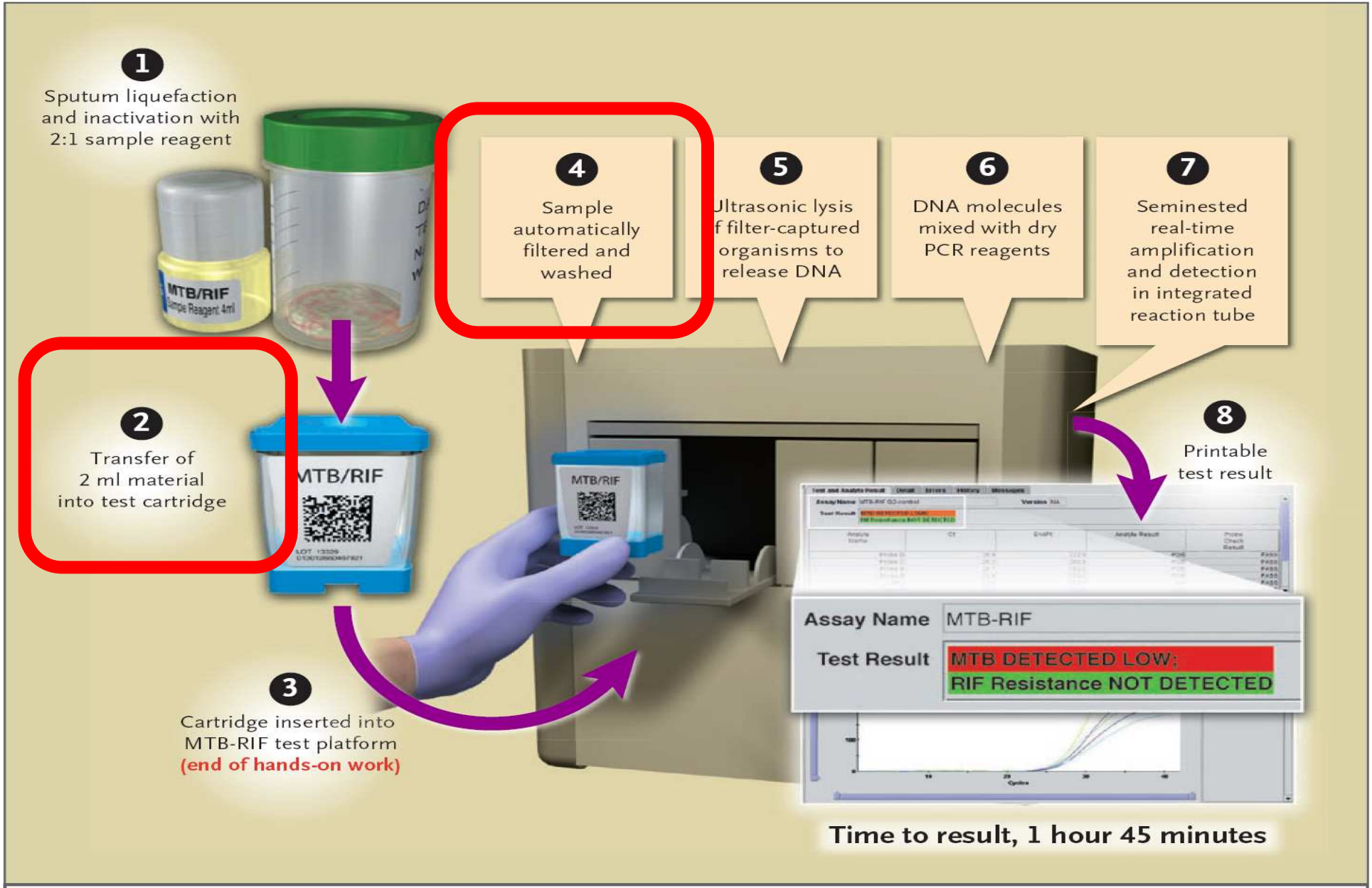


Figure 2. Assay Procedure for the MTB/RIF Test.

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.

Advantages

- Simple to perform
- Minimal training
- Virtually cross contamination free
- Minimal biosafety requirement
- Higher sensitivity in paucibacillary samples (HIV+)

Potential limits of Xpert MTB/RIF technology

- Unknown the performance at a district level
- If RFP resistance is diagnosed at a low level MDR prevalence environment, the assay needs to be confirmed
- Need to perform a culture for DST to evaluate other drug resistance
- Need to perform a culture for monitoring issue (culture conversion)

Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drug-resistant tuberculosis cases.

Migliori GB, et al. Eur Resp J. 2008 Jun;31(6):1155-9

TABLE 1 Outcomes of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) cases resistant and susceptible to injectable second-line drugs in Estonia, Germany, Italy and the Russian Federation (Archangels Oblast)[#]

Outcome	MDR-TB			XDR-TB		
	Capreomycin	Kanamycin	Amikacin	Capreomycin	Kanamycin	Amikacin
Treatment success						
Resistant	9 (39)	72 (66)	15 (72)	4 (36)	22 (48)	8 (36)
Susceptible	156 (72)	93 (71)	150 (69)	18 (49)	0 (0)	14 (54)
Died						
Resistant	5 (22)	20 (18)	3 (14)	4 (36)	12 (26)	8 (36)
Susceptible	38 (17)	23 (18)	40 (18)	10 (27)	2 (100)	6 (23)
Failure						
Resistant	9 (39)	18 (16)	3 (14)	3 (28)	12 (26)	6 (28)
Susceptible	23 (11)	14 (11)	29 (13)	9 (24)	0 (0)	6 (23)
Total						
Resistant	23 (100)	110 (100)	21 (100)	11 (100)	46 (100)	22 (100)
Susceptible	217 (100)	130 (100)	219 (100)	37 (100)	2 (100)	26 (100)

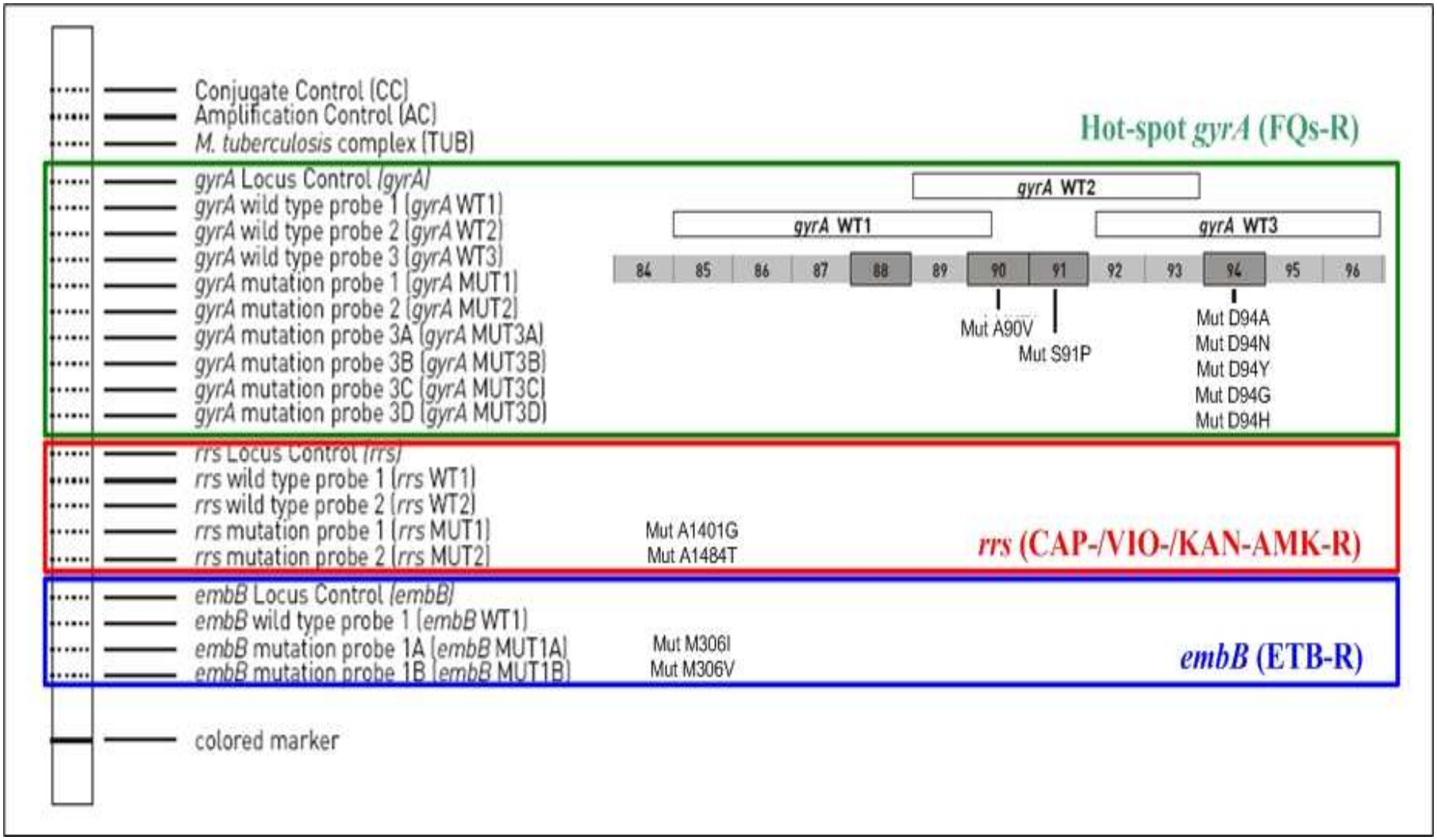
Data are presented as n (%). The percentage was calculated using the total number of cases resistant or susceptible to the given drug as a denominator. [#]: includes cases resistant to one and more than one injectable.

Geni coinvolti nella resistanza ai maggiori farmaci anti-tubercolari

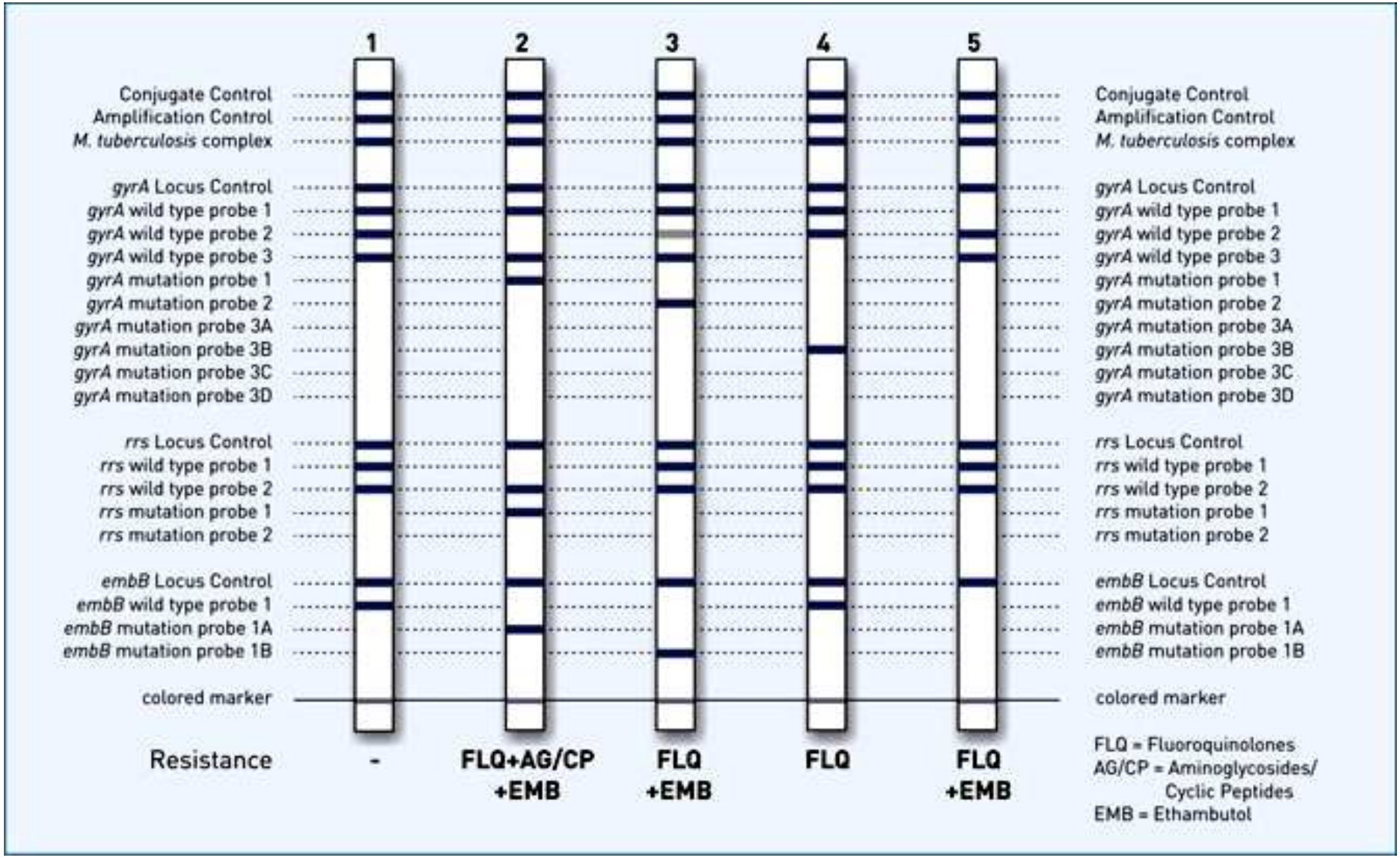
Drug	Gene	Gene product	Mutations
Streptomycin	*rpsL*	12S ribosomal protein	Coding region (60%)
	rrs	16S rRNA	Reg. 530 and reg. 915 (8%)
Isoniazid	*katG*	Catalase-peroxidase	coding region (cod. 315 - 60-80%)
	inhA	NADH-dep enoyl-ACP red	promoter reg. (Ribosome binding site - 15%); coding region
	ndh	NADH dehydrogenase	coding region
	ahpC-OxyR	regulon (controls *katG* and several other gene	promoter region (mutations relatively rare)
Rifampin	*rpoB*	RNA pol (β subunit)	hot spot region (cod. from 508 to 535 - 98%); N-term region
Ethambutol	*embB*	Arabinosyl transferase	ERDR (cod. 306 - 70%)
	embC	Arabinosyl transferase	coding region
Ethionamide	*inhA*	NADH-dep enoyl-ACP red	promoter reg. (Ribosome binding site); coding region
	ethA	Monooxygenase	coding region
	ethR	Monooxygenase repressor	coding region
	ndh	NADH dehydrogenase	coding region
Pyrazinamide	*pncA*	Pyrazinamidase	coding region (70%)
Fluoroquinolones	*gyrA*	DNA gyrase (sub. A)	QRDR (70%)
	gyrB	DNA gyrase (sub. B)	QRDR
Rifabutin	*rpoB*	RNA pol (β subunit)	coding region
Capreomycin	*rrs*	16S rRNA	coding region
	tlyA	rRNA methyltransferase	coding region
Viomycin	*rrs*	16S rRNA	coding region
Kanamycin	*rrs*	16S rRNA	coding region
Amikacin	*rrs*	16S rRNA	coding region
D-Cycloserine	*alr*	D-Ala racemase	promoter region
Para-salicylic acid	*thyA*	Thymidylate synthase	coding region

40-80%

GenoType® MTBDRs*l*



GenoType® MTBDRs*l*



Come interpretare i risultati dei tests

- Training nell'interpretazione del risultato:
- Refertare sensibile o resistente o il pattern di mutazione?
- Interpretare le discrepanze

GenoType MTBDR*plus* Assay for Molecular Detection of Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis* Strains and Clinical Samples

Lacoma A, et al. J Clin Microbiol. 2008 Nov;46(11):3660-7

TABLE 3. Distribution of MTBDR*plus* assay results according to the sequencing results for *katG*, *inhA*, and *oxyR-aphC* for the 48 INH^r strains

MTBDR*plus* test result	No. of the following INH^r strains with the indicated sequencing results:								
	Low-level INH^r (MICs ≤ 1 μg/ml)					High-level INH^r (MICs > 1 μg/ml)[a]			
	katG mutation	*inhA* mutation	*oxyR-ahpC* mutation	Wild type	Total	*katG* mutation	*inhA* mutation	Wild type	Total
INH^r	1	16	0	0	17	17	1	0	18
INH^s	2[b]	1[c]	1[d]	6	10	3[e]	0	0	3
Total	3	17	1	6	27	20	1	0	21

[a] None of the strains had the wild-type sequence.

[b] Both mutations were outside the *katG* hot-spot region studied by the MTBDR*plus* assay.

[c] The MTBDR*plus* assay did not identify a C→T *inhA* mutation at position −15 found by sequencing.

[d] This strain also had a Trp728Tyr change in *katG*.

[e] Two of the three strains had mutations outside the *katG* hot-spot region studied by the MTBDR*plus* assay. The other strain had a S315T mutation that was not detected by the MTBDR*plus* assay.


GRAZIE
PER
L'ATTENZIONE

ERROR: undefined
OFFENDING COMMAND: SCARPARO_Le

STACK:

(10)
/Title
()
/Subject
(D:20110128105616+01’00’)
/ModDate
()
/Keywords
(PDFCreator Version 0.9.5)
/Creator
(D:20110128105616+01’00’)
/CreationDate
(5317841)
/Author
-mark-