



3º CONGRESSO NEWMICRO
Padenghe sul Garda (BS), 20-22 Marzo 2013
..... the need for speed: il laboratorio di
Microbiologia e le urgenze infettive



Le nuove tecnologie per la gestione dell' urgenza/emergenza in Microbiologia:

Diagnosi rapida di TBC

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Diagnosi rapida di TBC

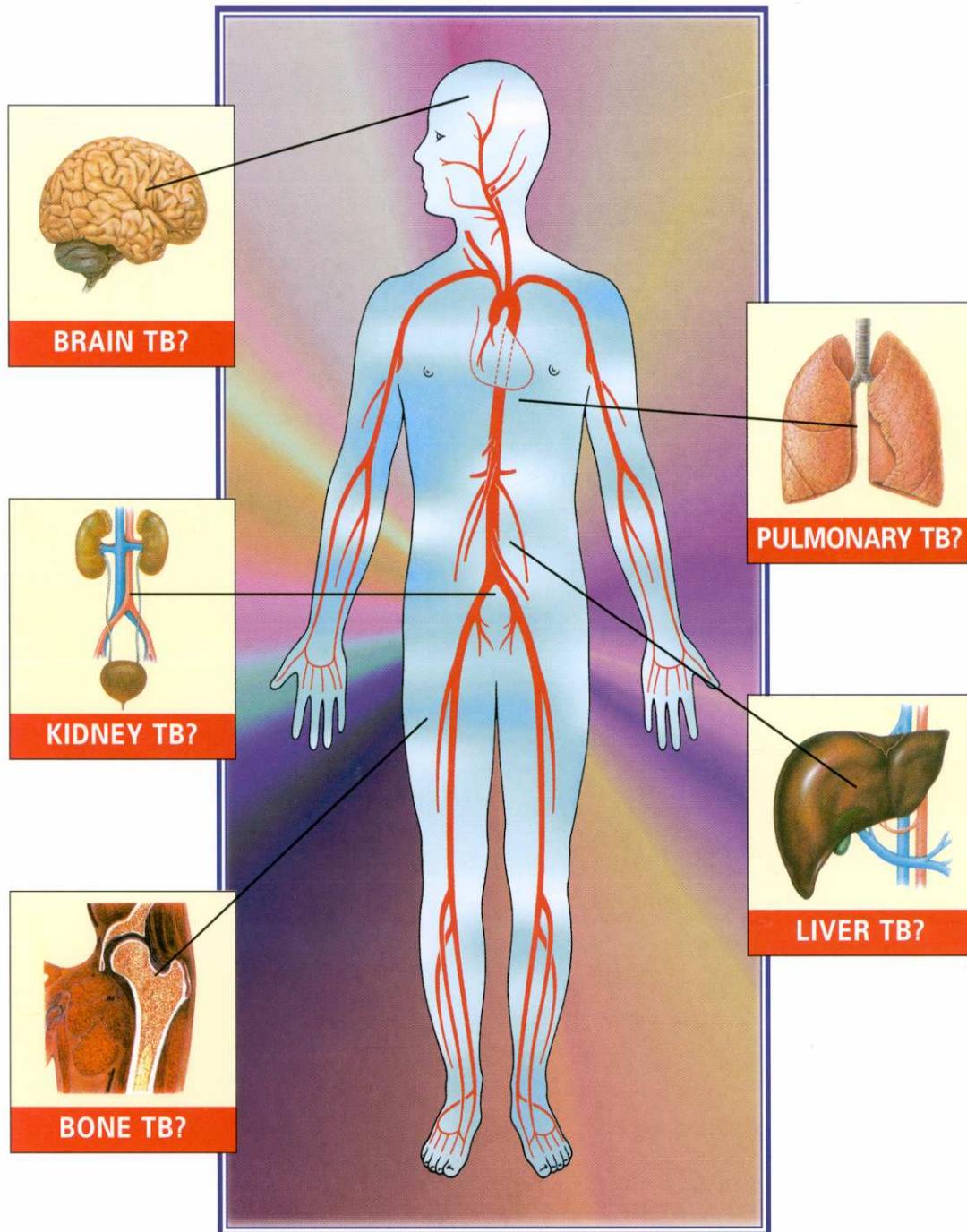
- a. Rapido isolamento del soggetto con espettorato microscopicamente positivo per evitare la diffusione della malattia.**
- b. Rapido e corretto approccio terapeutico con i farmaci di prima linea (possibile presenza di ceppi MDR).**
- c. Rapida e corretta gestione del soggetto e della sua terapia in presenza di ceppi XDR.**

Esame
microscopico

Rilevazione
direttamente
da materiale
biologico

Rilevazione
mutazioni
responsabili di
farmacoresistenza

IGRA:
InterFeron- γ
Release
Assay



TB



OR NOT

TB

PATOGENI CONVENZIONALI

Mycobacterium tuberculosis complex:

M. tuberculosis (hominis)

M. bovis

M. bovis BCG

M. africanum

M. canettii

M. microti

M. caprae

M. pinnipedii



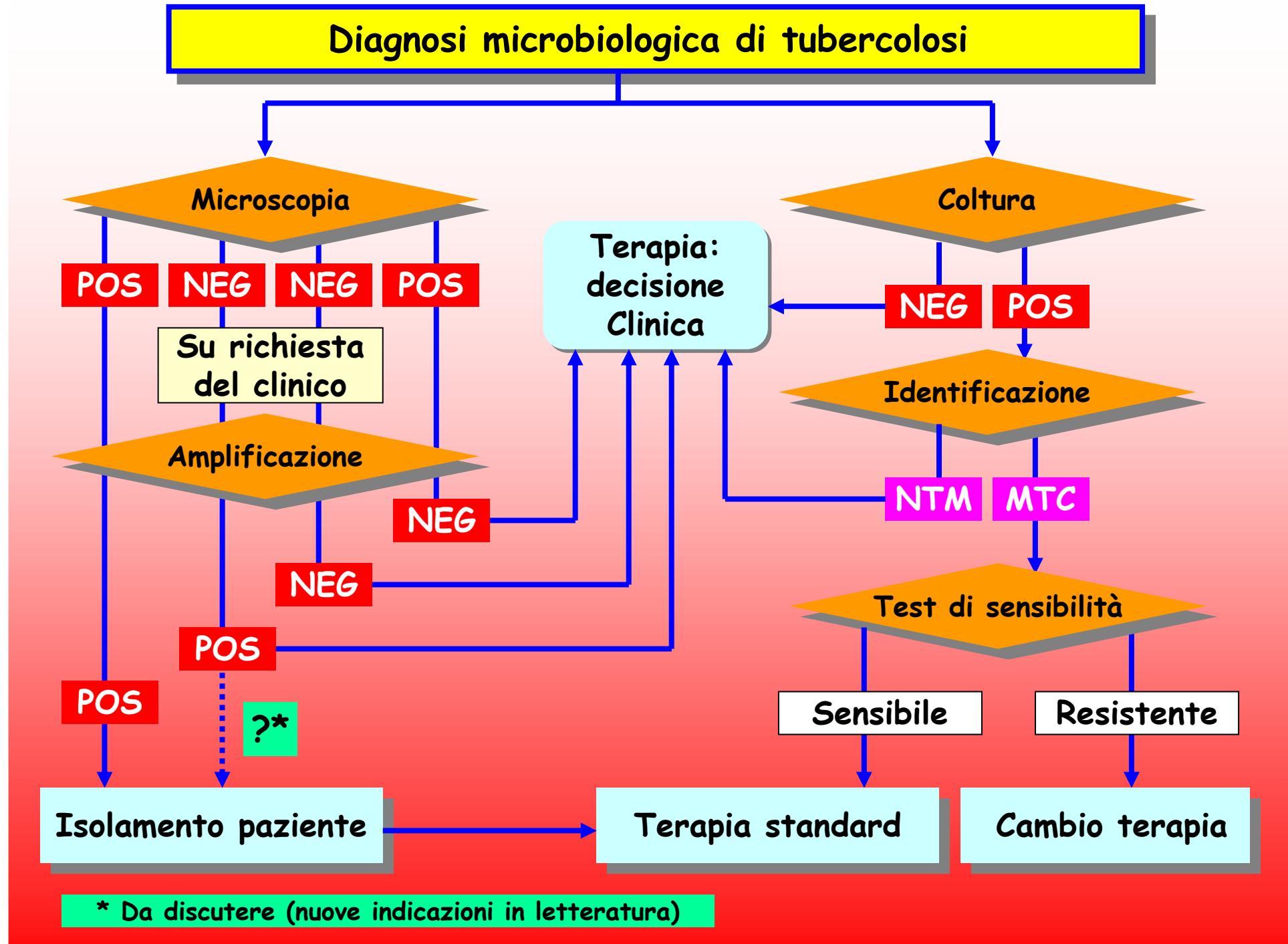
La richiesta di indagini microbiologiche per la ricerca di micobatteri

non è una procedura di screening

e pertanto deve essere avviata solo per quei pazienti in cui esista

un fondato sospetto clinico di tubercolosi

Tipo di campione	Requisiti	Istruzioni speciali	Campioni non idonei
Aspirato gastrico	≥ 5-10 mL raccolto al mattino, dopo almeno 8 ore di digiuno, per 3 giorni consecutivi	neutralizzare (pH 7) con carbonato di sodio	campioni non neutralizzati
Broncoaspirato, lavaggio bronco-alveolare, spazzolatura bronchiale, aspirato trans-tracheale	≥ 3 mL	disinfettare accuratamente il broncoscopio	
Espettorato	5-10 mL raccolto al mattino da espettorazione profonda, per 3 giorni consecutivi	istruire il paziente su come espettorare correttamente	saliva; pool di campioni
Espettorato indotto	5-10 mL raccolto al mattino, per 3 giorni consecutivi	specificare chiaramente nella richiesta, e sul contenitore, che si tratta di espettorato indotto	
Feci	almeno 1 g in contenitore senza conservanti	per la diagnosi di tubercolosi intestinale ricorrere al prelievo bioptico	
Linfonodo	linfonodo o porzione di esso in contenitore sterile, senza fissativi o conservanti	aggiungere una piccola quantità di fisiologica sterile	campioni in formalina o altri fissativi; campioni inclusi in paraffina
Liquidi cavitari: pleurico, pericardico, peritoneale ecc.	10-15 mL in provetta sterile (con eparina o citrato)	per la diagnosi di pleurite tubercolare sono più indicati biopsia pleurica ed espettorato indotto	
Liquor	≥ 2 mL		



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

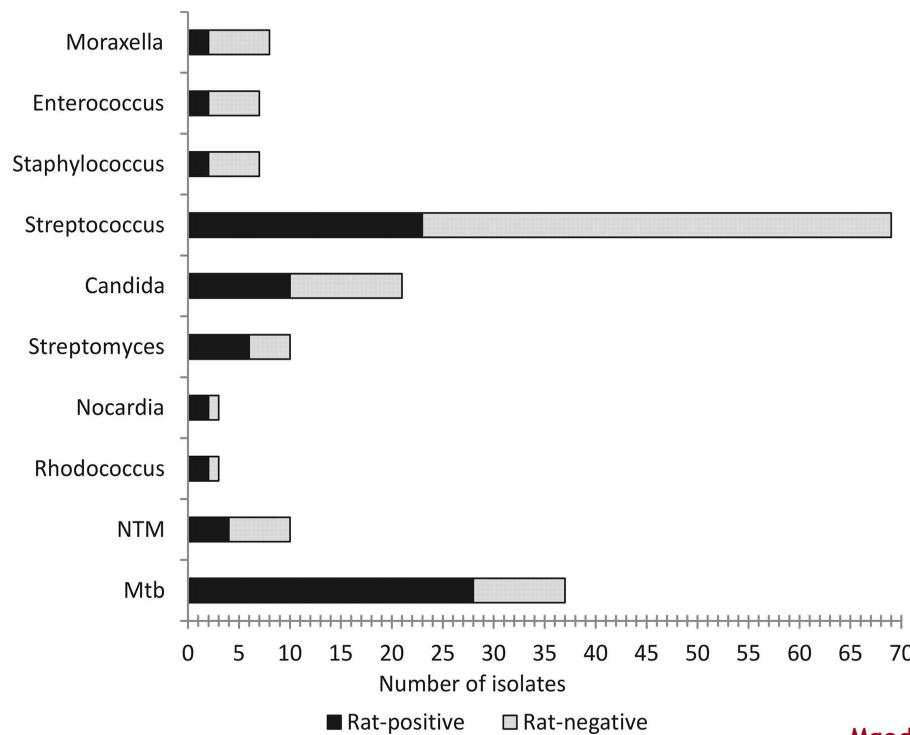
TABLE 3. ESSENTIAL LABORATORY TESTS FOR TUBERCULOSIS CONTROL

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

Journal of Clinical Microbiology



Cricetomys gambianus

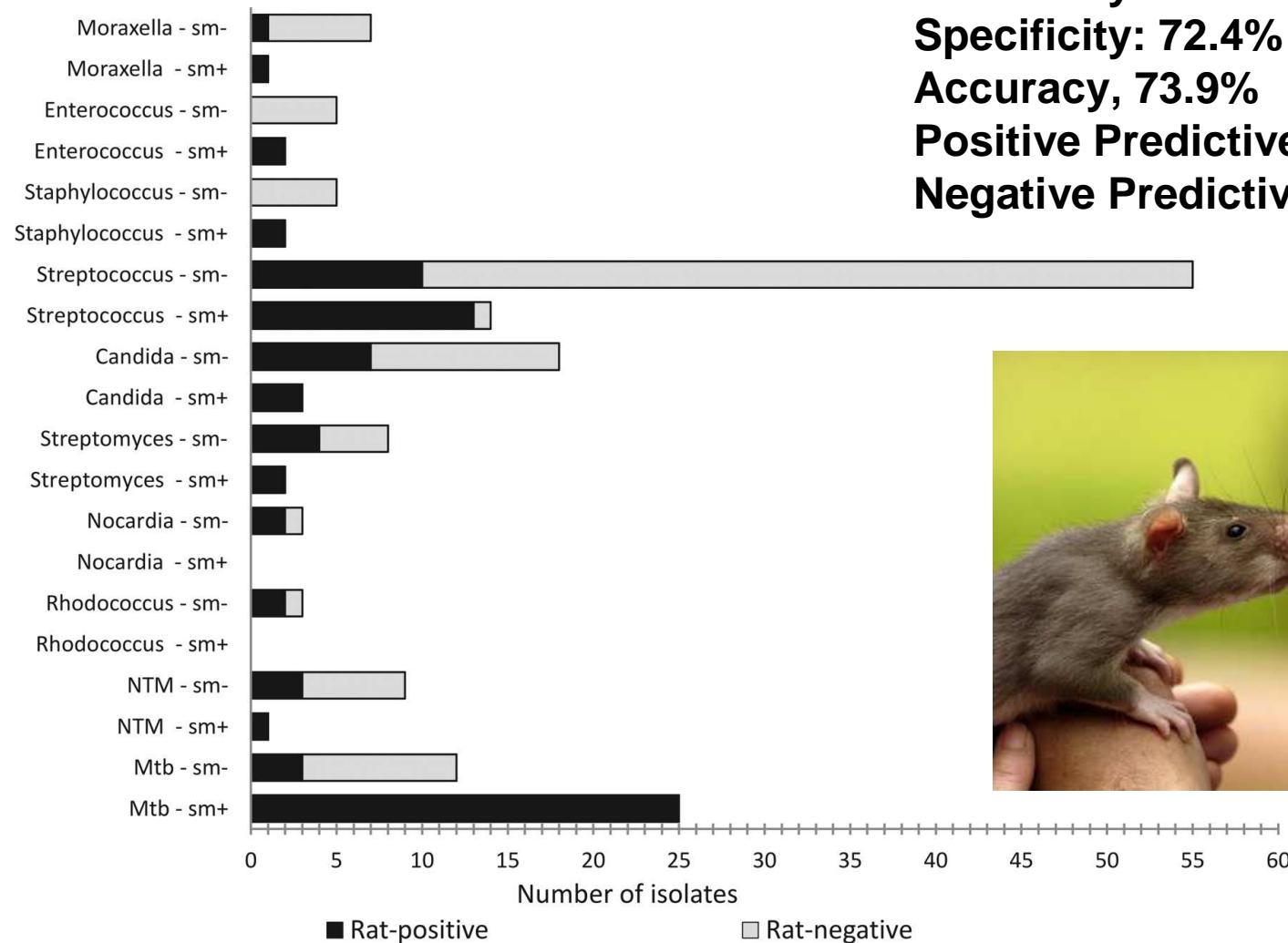


Diagnosis of Tuberculosis by Trained African Giant Pouched Rats and Confounding Impact of Pathogens and Microflora of the Respiratory Tract



70 campioni esaminati da 2 ratti
in 32 minuti

Patterns of rat positive and rat-negative in smear-positive (sm+) and smear negative (sm-) sputum samples with Mycobacterium and nonmycobacterial microorganisms.

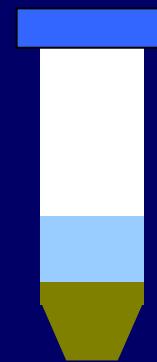


Sensitivity: 80.4%
Specificity: 72.4%
Accuracy, 73.9%
Positive Predictive Value: 41.7%
Negative Predictive Value: 93.8%

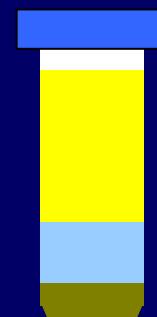


FLUIDIFICAZIONE E DECONTAMINAZIONE

NALC-NaOH 2% - metodo standard



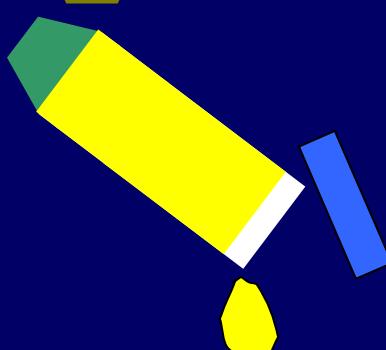
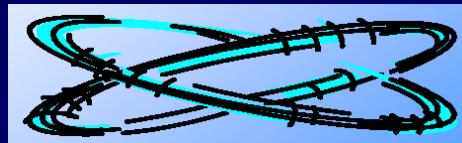
NALC-NaOH 2%
Campione



Tampone fosfato
0.067M a pH 6,8
NALC-NaOH 2%
Campione

Vortexare non più
di 30 sec.

Centrifugare
 $3800 \times g$ a $4^{\circ}C$



Decantazione

SEDIMENTO

15
minuti

15
minuti



Es. microscopico

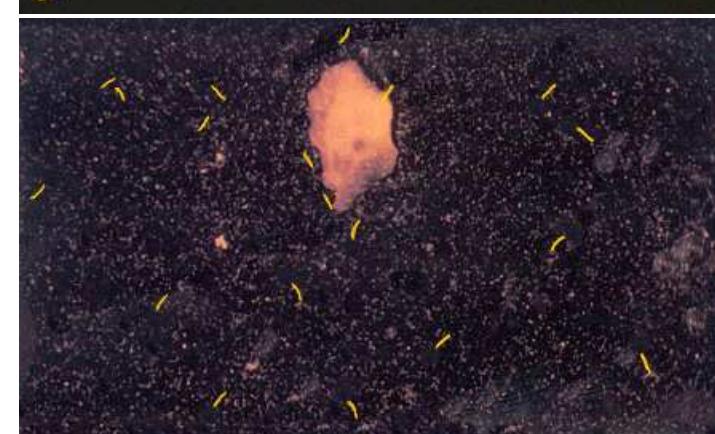
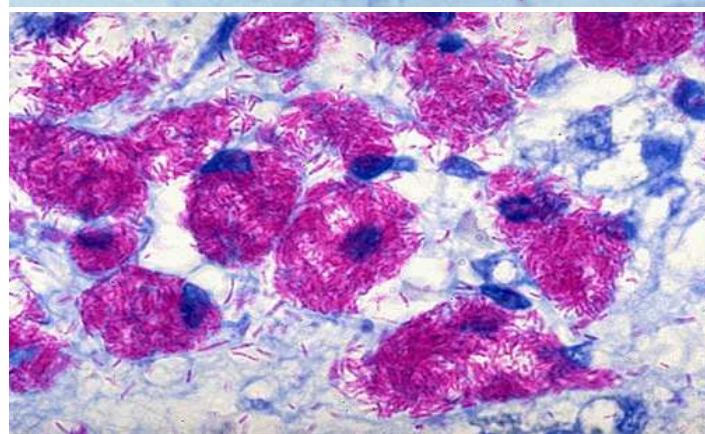
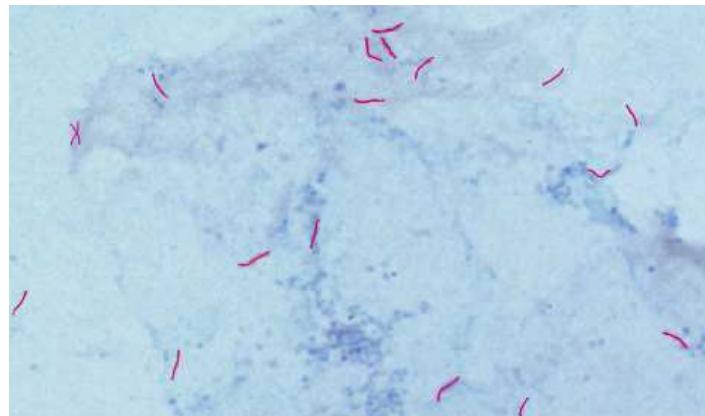
Es. colturale

T. amplificazione

ESAME MICROSCOPICO

Ziehl-Neelsen

Fluorescenza



Ob. 100 x - 1 vetrino in 15 minuti

Ob. 20 x - 1 vetrino in 2 minuti

Limite: 5.000 - 10.000 micobatteri/ml

Sensibilità 40 - 60 %

Int J Tuberc Lung Dis. 2003 Apr;7(4):376-81.
A comparison of direct microscopy, the concentration method and the Mycobacteria Growth Indicator Tube for the examination of sputum for acid-fast bacilli.

Apers L, Mutsvangwa J, Magwenzi J, Chigara N, Butterworth A, Mason P, Van der Stuyft P.
Department of Epidemiology, Provincial Medical Directorate, Epidemiology, Gweru,
Midlands, Zimbabwe.

In countries with high human immunodeficiency virus prevalence, laboratory diagnosis of pulmonary tuberculosis with the standard Ziehl-Neelsen (ZN) technique is characterised by low sensitivity.
DESIGN: Three hundred specimens from patients diagnosed with pulmonary tuberculosis were tested for the presence of mycobacteria. Specimens were stained with ZN, decontaminated by adding 4% NaOH, concentrated by centrifuging and processed in MGIT broth.

Decontamination and concentration of the sample increased the sensitivity of direct microscopy from 67.5% to 87.1%. Specificity remained unchanged (95.5%).

AFB smear positivity results for specimens culture positive for *M. tuberculosis* respiratory disease

Reference	Smear Method	Total no. of Respiratory Specimens	No. of Specimens Smear + / No. of Specimens (%) Culture +
Wright et al. JCM 1998	FL	6,532	677/1,082 (62.6)
Anargyros et al. JCM 1990	FL	2,563	46/162 (28.4)
Lipsky et al. Rev Infect Dis 1984	ZN	3,207	57/95 (60.0)
Levy et al. Chest 1989	ZN	2,560	71/107 (66.3)
Strumpf et al. Am Rev Resp Dis 1979	FL	1,769	70/106 (66.0)
Gordin and Slutkin Arch Pathol Lab Med 1990	FL	2,956	137/264 (51.9)
Yajko et al Clin Infect Dis 1994	FL	19,343	248/450 (55.1)
Badak et al. Gen. Meeting ASM 1995	FL	16,336	97/157 (62.5)
Murray et al. Am Intern Med 1980	FL	10,468^a	(46)

a: For all mycobacteria, 158 of 406 (38.9%) were fluorochrome positive

I test di amplificazione diretta (DAT)

consentono la simultanea rilevazione ed identificazione del *M. tuberculosis complex* direttamente dai campioni clinici, con risultati disponibili nel giro di poche ore.

Raccomandazioni dei *CDC* MMWR 2009; 58: 7-10 sull'impiego del test di amplificazione

- 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**

I sistemi commerciali (1)

Sistema	E-MTD	Amplicor	ProbeTec
Metodo	TMA	PCR	SDA
Bersaglio	rRNA	rDNA	IS 6110
Lettura	Chemioluminescenza	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)

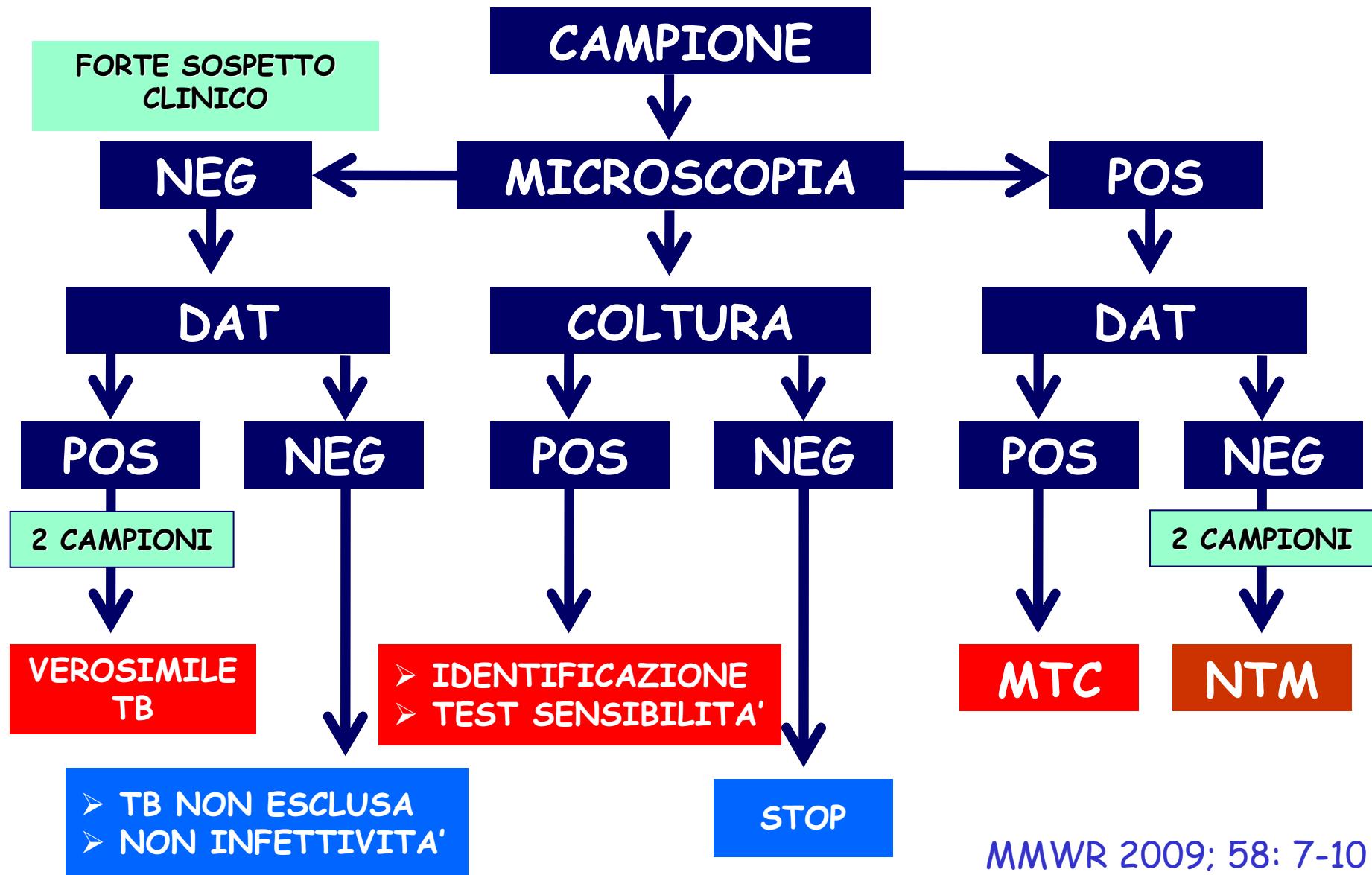
Sistema	Real Art MTB PCR	MTB Q PCR Alert	COBAS TaqMan	DUPLICa MTB
Metodo	qRT-PCR	RT-PCR	RT-PCR	RT-PCR
Target	16S rDNA	IS 6110	16S rDNA	16S-23S rDNA
Sonda	TaqMan	TaqMan	TaqMan	TaqMan
C.I.A.	eterologo	eterologo	omologo	omologo
Estrazione	Lisi- cattura	Lisi- cattura	Lisi	Lisi- cattura
Sensibilità (n. di 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

Diagnosis of tuberculosis and drug resistance: what can new tools bring us?

Drobniowski F., et al. Int J Tuberc Lung Dis 2012; 16(7):860-870

Table 1 Commercially available NAA assays for tuberculosis detection in clinical specimens*

Assay	Manufacturer	Method	Material	Sensitivity % (95%CI) [†]	Specificity % (95%CI) [†]
Amplified MTD	Gen-Probe Inc, San Diego, CA, USA	Transcription-mediated amplification of RNA	DNA from decontaminated sputum	86.0 (74.2–93.7)	99.3 (96.3–100.0)
COBAS® TaqMan® MTB Test	Roche Molecular Diagnostics, Pleasanton, CA, USA	RT-PCR	DNA from decontaminated sputum	91.5 (86.9–96.1)	98.7 (98.0–99.4)
<i>artus® M. tuberculosis</i> PCR	Qiagen, Hilden, Germany	RT-PCR	DNA from decontaminated sputum	79.1	98.2
Loopamp® Tuberculosis Complex Detection Reagent Kit	Eiken Chemical, Tokyo, Japan	LAMP	Untreated sputum	97.8 (93.6–95.5)	85.1 (75.8–91.8)
Amplicor MTB	Roche Molecular Diagnostics	PCR amplification of 16S RNA	DNA from decontaminated sputum	—	—
Cobas Amplicor	Roche Molecular Diagnostics	PCR amplification of 16S RNA	DNA from decontaminated sputum	—	—
LCx	Abbott Laboratories, Abbott Park, IL, USA	Ligase chain reaction amplification of 38kDa protein	DNA from decontaminated sputum	88.9 (82.5–96.3)	96.8 (95.1–98.5)
BD Probe Tec Direct	BD, Sparks, MD, USA	Strand displacement amplification of IS6110 and 16S RNA	DNA from decontaminated sputum	77.5 (72.0–83.0)	98.0 (97.1–98.9)

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+			AFB-		
		DOR	Sensitivity	Specificity	DOR	Sensitivity	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, transcription 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

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

*: Cobas Amplicor Roche the three groups of patients analyzed

Clinical suspicion	Patient group ^d	% Positive (no. of positive sample results/total no. tested) by:		
		<i>M. tuberculosis</i> 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**

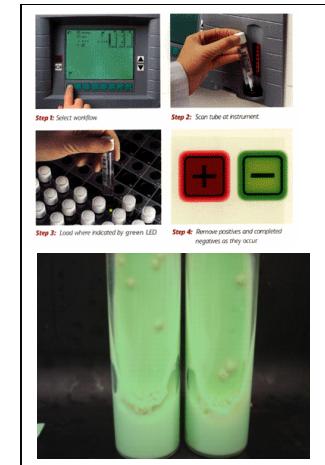
Falsi positivi

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

Susceptibility Testing in *M. tuberculosis*

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

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



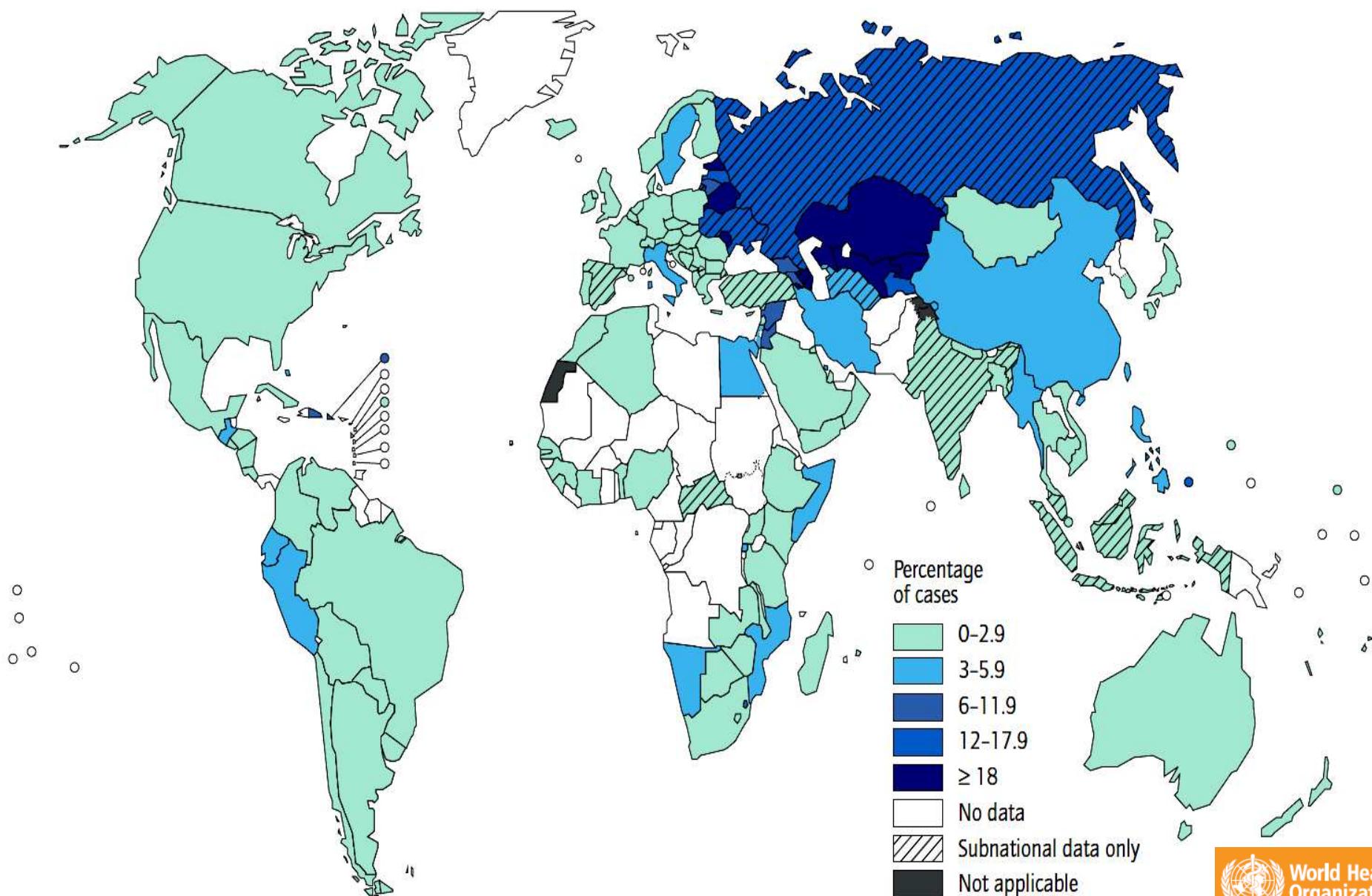
Metodi Molecolari (identificazione delle mutazioni responsabili di resistenza):

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



FIGURE 4.2 Percentage of new TB cases with MDR-TB^a

Drug-resistant TB



^a Figures are based on the most recent year for which data have been reported, which varies among countries.

FIGURE 4.3 Percentage of previously treated TB cases with MDR-TB^a

Drug-resistant TB

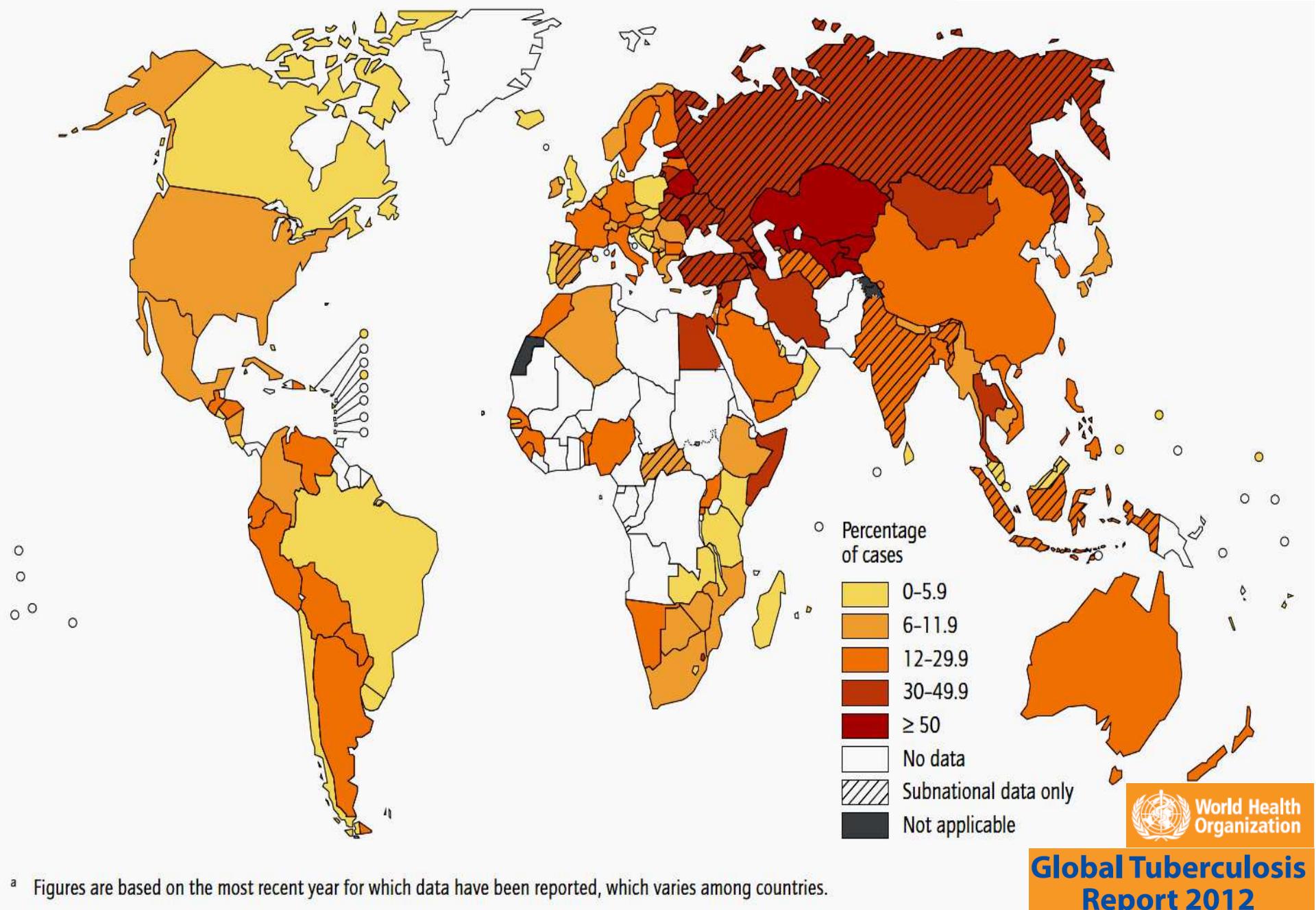
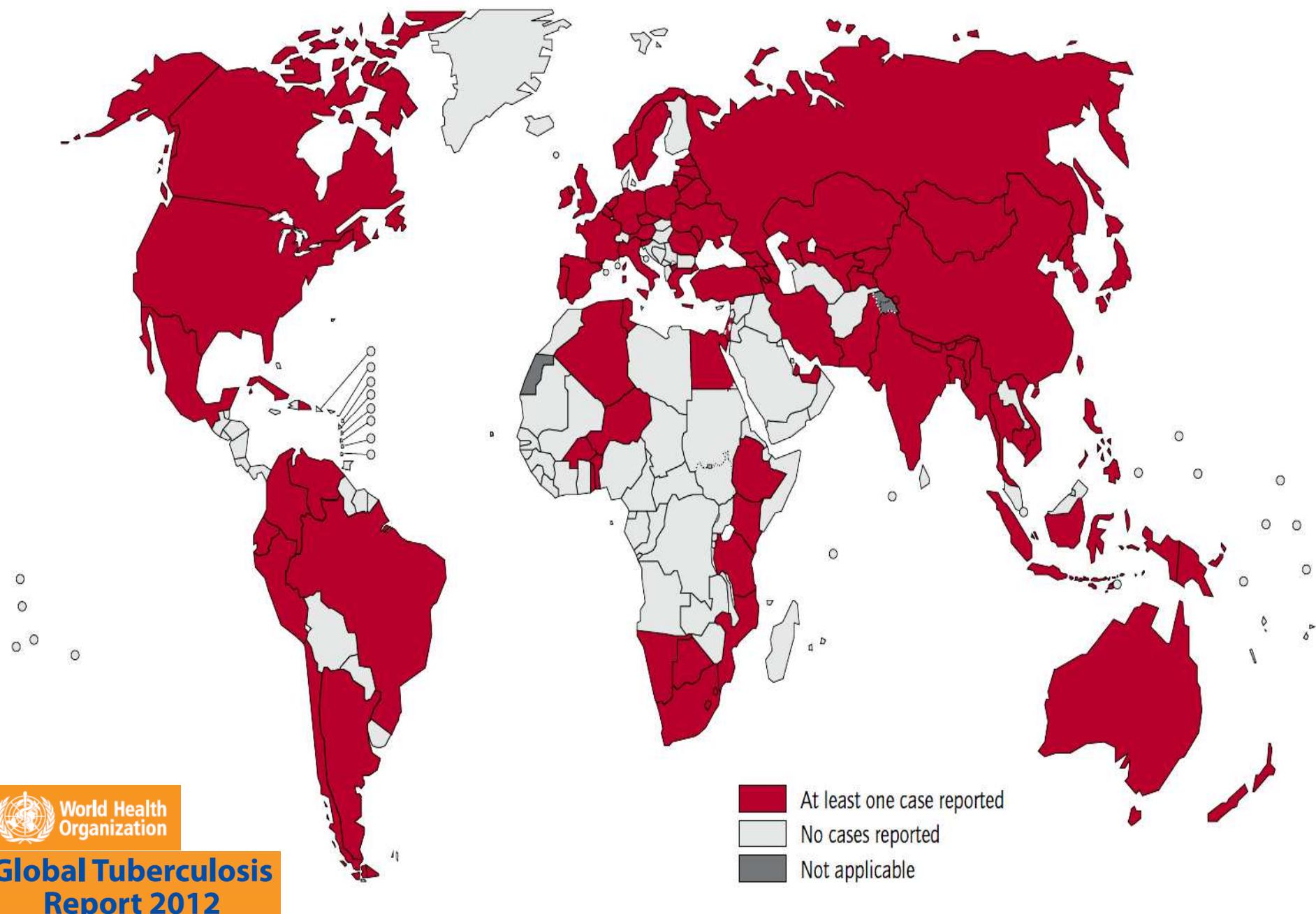


FIGURE 4.4 Countries that had notified at least one case of XDR-TB by the end of 2011 **Drug-resistant TB**



MULTI DRUG-RESISTANT (MDR) TB

Resistenza ad almeno isoniazide e rifampicina

EXTENSIVELY DRUG-RESISTANT (XDR) TB

MDR + resistenza ai fluorochinoloni ed almeno un farmaco di seconda linea iniettabile

Farmaci di seconda linea per il trattamento di MDR TB

Aminoglicosidi

Amikacina, Kanamicina

}

“Iniettabili”

Polipeptidi

Capreomicina

Fluorochinoloni

Ciprofloxacin, Ofloxacin, Moxifloxacin

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, microaerofilia, 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 resistenza ai maggiori farmaci anti-tuberculari

rpoB

Drug Gene Gene product Mutations

Streptomycin	<i>rpsL</i>	12S ribosomal protein	Coding region (60%)
Isoniazid			
Rifampicin			
Ethambutol			
Ethionamide			
Pyrazinamide			
Fluoroquinolones			
Rifabutin			
Capreomycin			
Viomycin			
Kanamycin	<i>rrs</i>	16S rRNA	coding region
Amikacin	<i>rrs</i>	16S rRNA	coding region
D-Cycloserine	<i>alr</i>	D-Ala racemase	promoter region
Para-salicylic acid	<i>thyA</i>	Thymidylate synthase	coding region

507 81 base pair core region 533

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

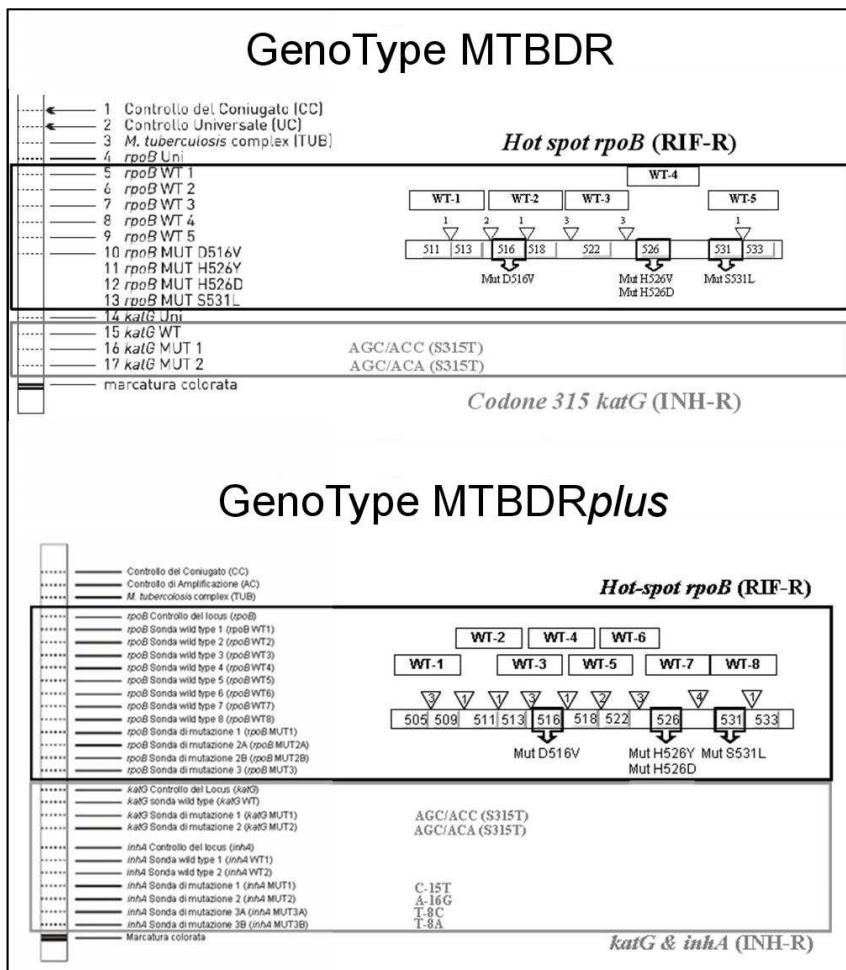
Ottimo candidato per la diagnosi molecolare di resistenza

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

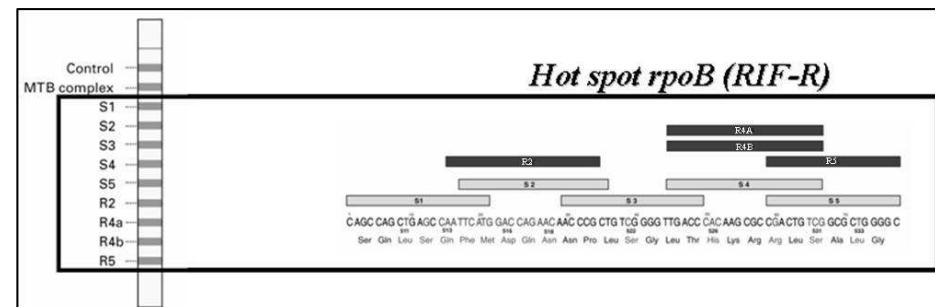
Test commerciali per la farmacoresistenza

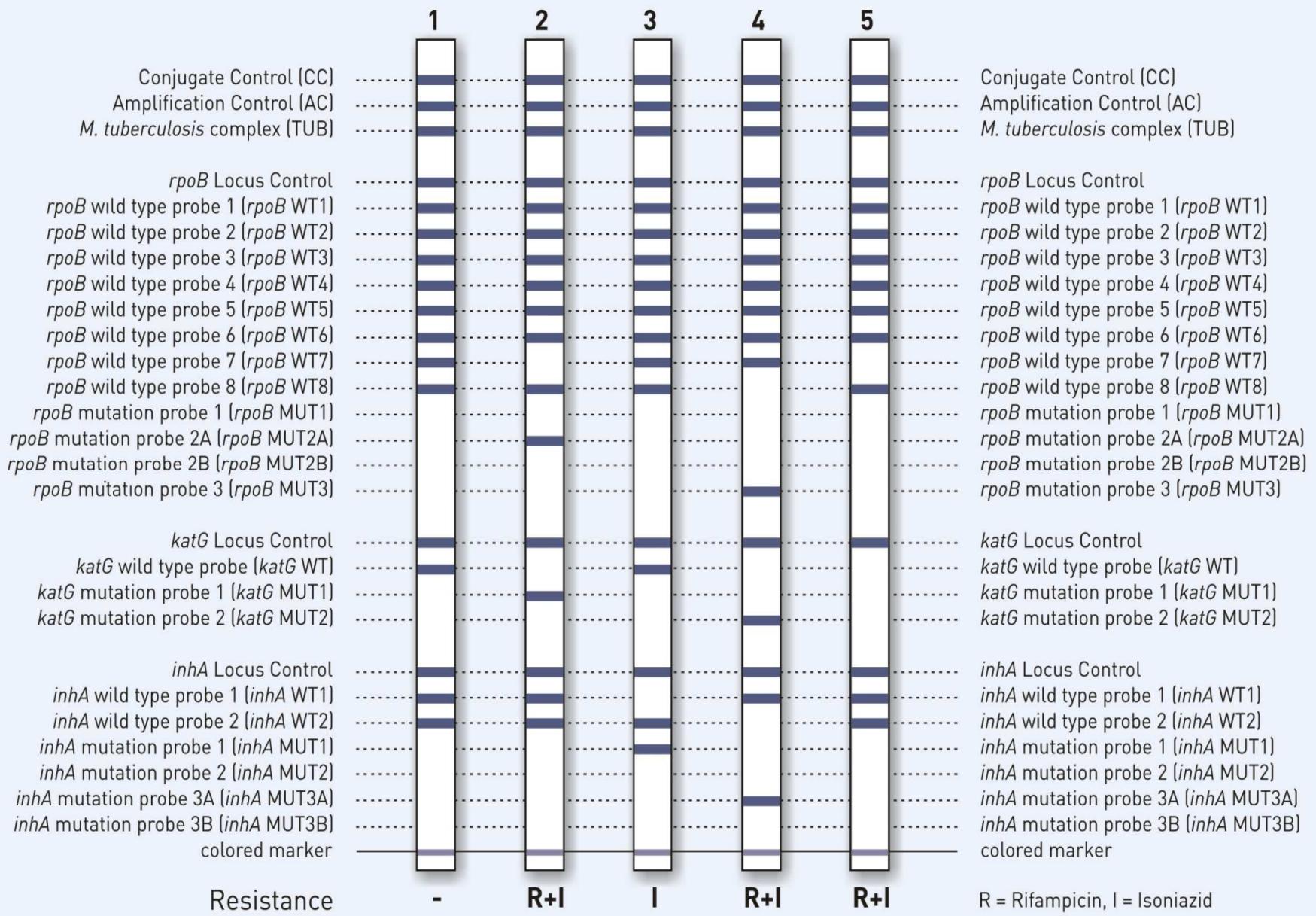
Hain Lifescience



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)

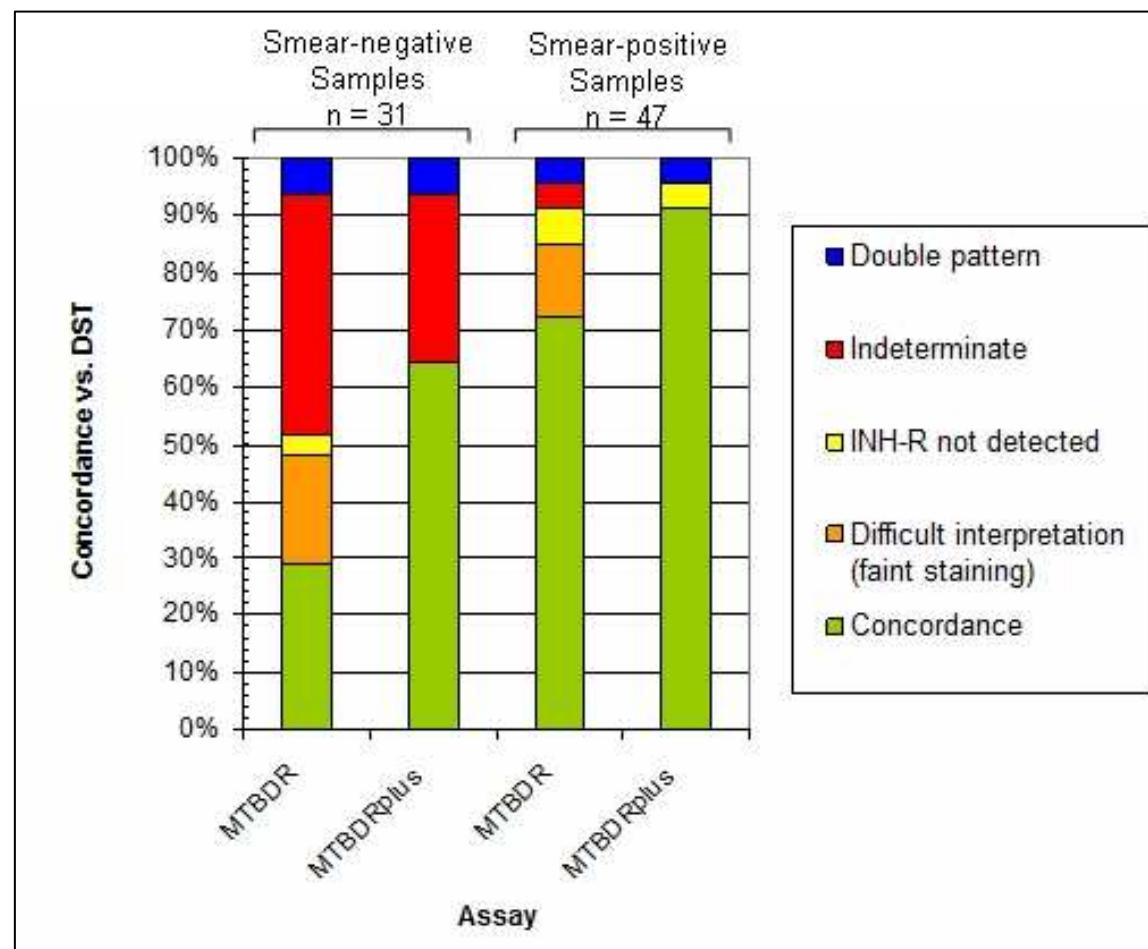
INNO-LIPA Rif. TB

Table I: 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 MTBDRplus: 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 MTBDRplus 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. MTBDRplus assay results according to Bactec 460TB system results for the 62 clinical strains

MTBDRplus test result	Bactec 460TB system result (no. [%] of strains)			
	INH		RIF	
Susceptible	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

The NEW ENGLAND
JOURNAL *of* MEDICINE

Rapid Molecular Detection of Tuberculosis
and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabetta, 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.

10.1056/NEJMv1008496 NEJM.ORG

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The New England Journal of Medicine

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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



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



South Africa
UCT
SAMRC

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



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

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

Performance of Nucleic Acid Amplification Tests for Diagnosis of Tuberculosis in a Large Urban Setting

Laraque F, et al. Clin Infect Dis. 2009; 49:46-54

Table 4. Performance of nucleic acid amplification (NAA) testing of patients' specimens obtained from body sites other than the respiratory tract, compared with culture positivity, in New York City during 2000–2004.

Type of specimen tested	No. of specimens ^a	Sensitivity, %	Specificity, %	PPV, %	NPV, %
All	682	89.3	74.5	79.3	86.5
Positive for AFB on smear	215	97.5	93.6	95.1	96.8
Negative for AFB on smear	383	83.2	65.6	70.7	79.7
Cerebrospinal fluid	188	84.9	62.1	68.7	80.8
Lymph node tissue	88	97.0	66.7	90.3	87.5
Gastric aspirate	65	100.0	90.0	95.7	100.0
Pleural fluid	56	100.0	87.5	76.2	100.0
Peritoneal fluid	31	92.3	77.8	75.0	93.3

NOTE. AFB, acid-fast bacilli; NPV, negative predictive value; PPV, positive predictive value.

^a Number of specimens that were tested for *Mycobacterium tuberculosis* on culture and by NAA.

Clinical validation of the Xpert MTB/RIF test for the diagnosis of tuberculosis in extrapulmonary samples in a low prevalence Country

Tortoli E., et al. Eur Respir J 2012; 40: 442-447

Samples	Number	a) Gold standard = MTC positive culture + clinical diagnosis			
		Sensitivity	Specificity	Positive LR	Negative LR
Biopsy specimens	368	88.3	100.0	∞	0.1
Pleural fluid	330	44.4	100.0	∞	0.6
Gastric aspirate	224	78.7	100.0	∞	0.2
Pus	195	87.3	100.0	∞	0.1
CSF	133	85.7	99.1	102.0	0.1
Urine	130	87.5	99.1	99.7	0.11
Cavitory fluid	94	50.0	100.0	∞	0.5
Total	1,474	81.3	99.8	490.5	0.2
Smear positive	127	99.3	95.8	23.8	0.0
Smear negative	1,347	70.3	99.9	831.0	0.3

Clinical validation of the Xpert MTB/RIF test for the diagnosis of tuberculosis in extrapulmonary samples in a low prevalence Country

Tortoli E., et al. Eur Respir J 2012; 40: 442-447

TABLE 1

Comparison of Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA) results with culture and clinical data

Sample type	Xpert-positive and MTC-positive culture	Xpert-negative and MTC-positive culture	Xpert-positive, MTC-negative culture and TB diagnosis	Xpert-negative NTM-positive culture	Xpert-positive, MTC-negative culture and non-TB diagnosis	Xpert-negative, MTC-negative culture	Xpert-indeterminate culture	Total (% proportion)
Biopsy specimen								368 (25.0)
Pleural fluid								330 (24.4)
Gastric aspirate								224 (15.2)
Pus	40	7	8	22	0	118	4	195 (13.2)
CSF	11	2	1	0	1	118	5	133 (9.0)
Urines	11	2	3	6	1	107	0	130 (8.8)
Cavitory fluid	5	5	0	0	0	84	0	94 (6.4)
Total	188	50	30	61	2	1143	17	1474 (100)

CSF: 5 campioni microscopici positivi

Advantages Xpert MTB/Rif

- 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)

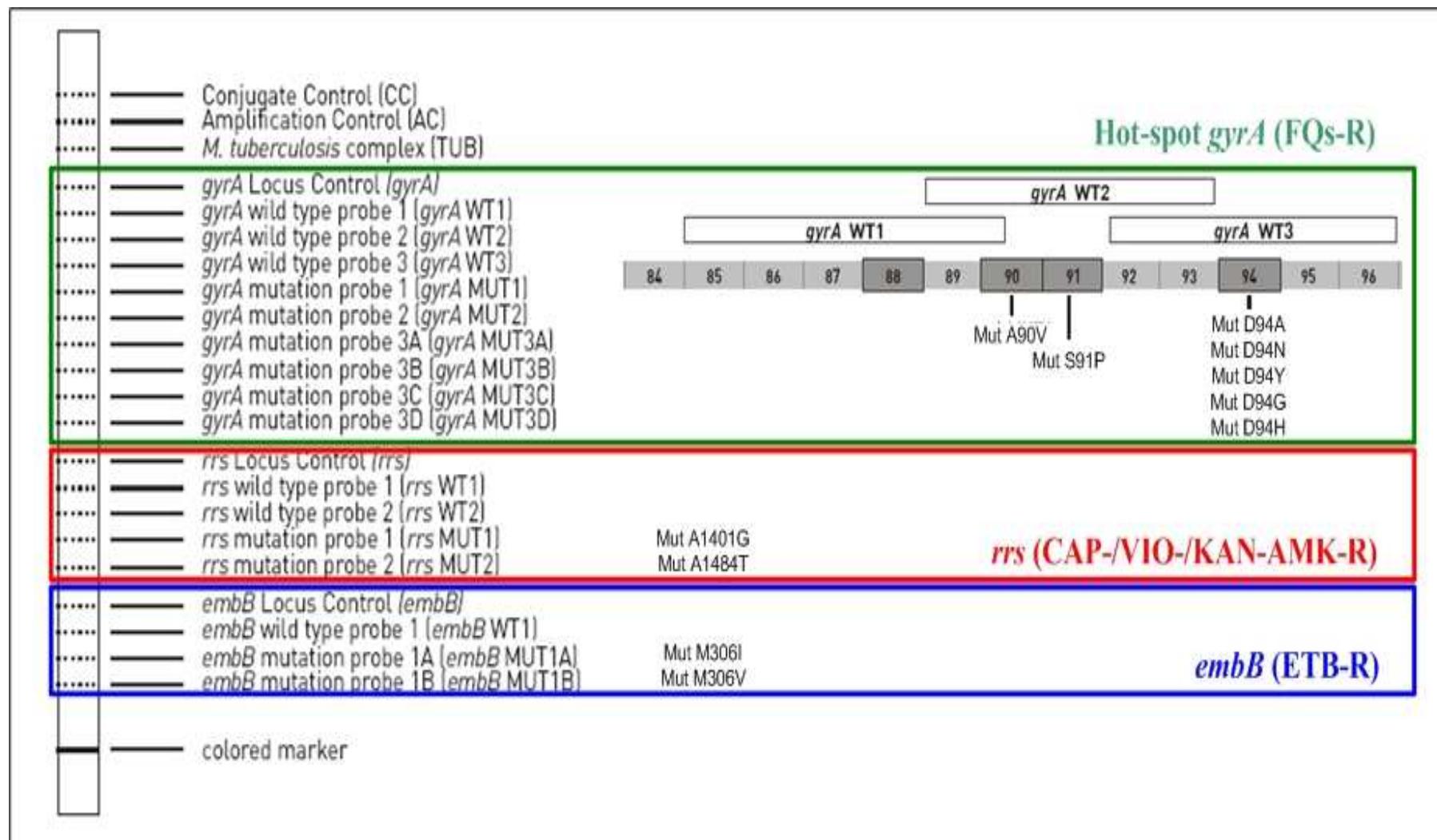
Data are presented as n (%). The percentage was calculated using the total number of cases resistant or susceptible to the given drug as a denominator. [#]: includes cases resistant to one and more than one injectable.

Geni coinvolti nella resistenza ai maggiori farmaci anti-tubercolari

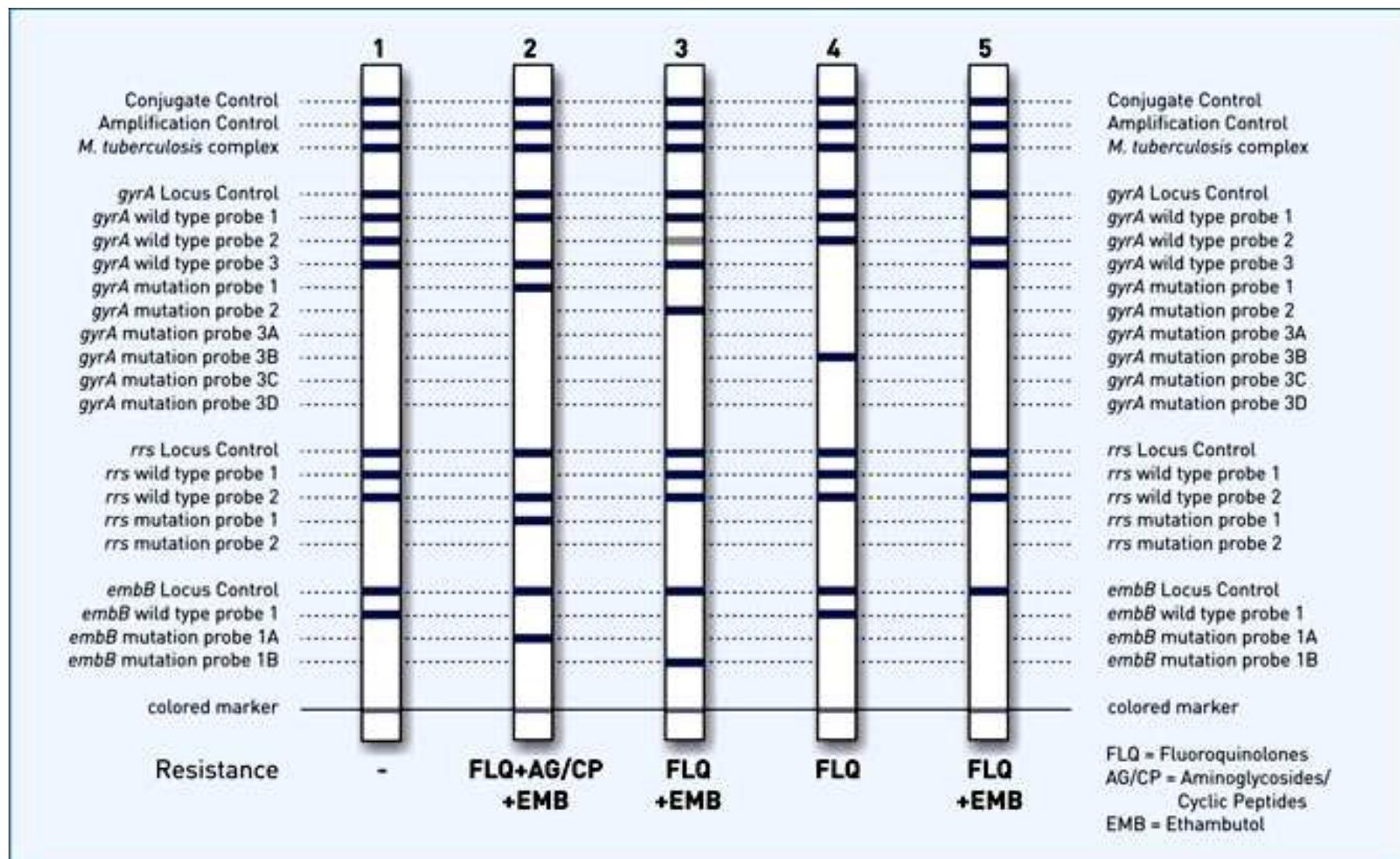
Drug	Gene	Gene product	Mutations
Streptomycin	<i>rpsL</i>	12S ribosomal protein	Coding region (60%)
	<i>rrs</i>	16S rRNA	Reg. 530 and reg. 915 (8%)
Isoniazid	<i>katG</i>	Catalase-peroxidase	coding region (cod. 315 - 60-80%)
	<i>inhA</i>	NADH-dep enoyl-ACP red	promoter reg. (Ribosome binding site - 15%);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

40-80%

GenoType® MTBDRsI



GenoType® MTBDRsI



GenoType MTBDRsl Line Probe Assay Shortens Time to Diagnosis of Extensively Drug-Resistant Tuberculosis in a High-Throughput Diagnostic Laboratory

Barnard M., et al. Am J Respir Crit Care Med 2012 Dec 15;186(12):1298-305

516 smear-positive pulmonary and extrapulmonary specimens

TABLE 1. PERFORMANCE OF GENOTYPE MTBDRs/ LINE PROBE ASSAY IN DETECTING OFLOXACIN AND AMIKACIN RESISTANCE AND EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS

	OFX % (95% CI)	AMK % (95% CI)	XDR-TB % (95% CI)
Sensitivity	90.7 (80.1–96.0)	100 (91.8–100)	92.3 (75.9–97.9)
Specificity	98.1 (96.3–99.0)	99.4 (98.2–99.8)	99.6 (98.5–99.9)
PPV	84.5 (73.1–91.6)	93.5 (82.5–97.8)	92.3 (75.9–97.9)
NPV	98.9 (97.5–99.5)	100 (99.2–100)	99.6 (98.5–99.9)
OCC	97.3 (95.5–98.4)	99.4 (98.3–99.8)	99.2 (98.0–99.7)
κ	86.0 (77.4–94.6)	96.3 (87.7–100)	91.9 (83.3–100)

Definition of abbreviations: AMK = amikacin; CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value; OCC = overall correct classification; OFX = ofloxacin; XDR-TB = extensively drug-resistant tuberculosis.

Rapid Diagnosis of Drug Resistance to Fluoroquinolones, Amikacin, Capreomycin, Kanamycin and Ethambutol Using Genotype MTBDRsI Assay: A Meta-Analysis

Feng Y, et al. PLoS One. 2013;8(2):e55292.

Drug resistance to fluoroquinolones

Pooled Sensitivity = 0.87 (0.85 to 0.90)

Pooled Specificity = 0.97 (0.96 to 0.98)

Drug resistance to amikacin

Pooled Sensitivity = 0.83 (0.78 to 0.87)

Pooled Specificity = 0.99 (0.99 to 1.00)

Drug resistance to capreomycin

Pooled Sensitivity = 0.82 (0.77 to 0.86)

Pooled Specificity = 0.97 (0.96 to 0.98)

Drug resistance to kanamycin

Pooled Sensitivity = 0.44 (0.40 to 0.49)

Pooled Specificity = 0.99 (0.99 to 1.00)

Drug resistance to ethambutol

Pooled Sensitivity = 0.68 (0.65 to 0.71)

Pooled Specificity = 0.80 (0.77 to 0.82)

The lack of data did not allow for proper evaluation of the test on clinical specimens.

Come interpretare i risultati dei tests

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

GenoType MTBDRplus 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 MTBDRplus assay results according to the sequencing results for *katG*, *inhA*, and *oxyR-aphC* for the 48 INH^r strains

MTBDRplus 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			
	<i>katG</i> mutation	<i>inhA</i> mutation	<i>oxyR-aphC</i> mutation	Wild type	Total	<i>katG</i> mutation	<i>inhA</i> 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 MTBDRplus assay.

^c The MTBDRplus 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 MTBDRplus assay. The other strain had a S315T mutation that was not detected by the MTBDRplus assay.

Anyplex™ II MTB/MDR/XDR Detection

Specimen

- Sputum
- Fresh Tissue
- Culture
- Bronchial washing

DNA Extraction **(1hr*)**



Real-time PCR and Detection **(3hr)**



*24 samples



Result Interpretation and Clinical Implication

Tube	Fluorophore	Melting temp. (Tm)	Corresponding gene / mutations	Result interpretation	Clinical implication
MTB/MDR MTB/XDR	FAM	Single Tm range	<i>mpb64</i>	MTB detected	MTB infection
	CAL Orange 560	Single Tm range	18 mutations in <i>rpoB</i>	RIF-R	RIF resistance
MTB/MDR	CAL Red 610	Low Tm	4 mutations in <i>katG</i>	INH-R	High-level INH resistance
		High Tm	3 mutations in <i>inhA</i> promoter	INH-R	Low-level INH resistance
MTB/XDR	CAL Orange 560	Single Tm range	7 mutations in <i>gyrA</i>	FQ-R	FQ resistance
	CAL Red 610	Low Tm	3 mutations in <i>eis</i> promoter	Injectable drug-R	Low-level KAN resistance
		Middle Tm	2 mutations in <i>rrs</i> (1401G/1484T)	Injectable drug-R	High-level KAN/AMII/CAP resistance
		High Tm	1 mutation in <i>rrs</i> (1402T)	Injectable drug-R	Low-level KAN resistance High-level CAP resistance

KAN, Kanamycin; AMI, Amikacin; and CAP, Capreomycin

Summary

- Highly informative product for the diagnosis and treatment of TB
Simultaneous screening of MTB infection and anti-TB drug resistance
- Product with extremely high specificity in drug resistance detection
Direct and positive-based detection of mutations causing anti-TB drug resistance
 - 7 mutations causing Isoniazid resistance (INH-R)
 - 18 mutations causing Rifampicin resistance (RIF-R)
 - 7 mutations causing fluoroquinolone resistance (FQ-R)
 - 6 mutations causing injectable drug resistance
- Prominent specificity and sensitivity by proprietary oligo technology (**DPOTM**) and real-time PCR chemistry (**TOCETM**)
- Simple-to-use system and simple-to-result interpretation system
- Comparably rapid system : Result in **4hrs**



TUBERCULIN SKIN TEST

INTRADERMOREAZIONE TUBERCOLINICA SECONDO MANTOUX

➤ Reagenti:

- ✓ Iniezione intradermica di 5UI di PPD (Purified Protein Derivative) comunemente presenti in differenti micobatteri (*M. tuberculosis*, *BCG* e micobatteri non tubercolari)

➤ Variabilità:

- ✓ Riproducibilità del test
- ✓ Soggettività nella lettura del test

➤ Logistica

- ✓ Necessità di 2 visite
- ✓ Risultati dopo 3 giorni



Possibili risultati: ????

TUBERCULIN SKIN TEST

INTRADERMOREAZIONE TUBERCOLINICA SECONDO MANTOUX

POSSIBILI RISULTATI	POSITIVO	≥5 mm	≥10 mm	≥15 mm
	NEGATIVO	<5 mm	<10 mm	<15 mm

Classification of the Tuberculin Skin Test Reaction

<p>An induration of 5 or more millimeters is considered positive in</p> <ul style="list-style-type: none"> • HIV-infected persons • A recent contact of a person with TB disease • Persons with fibrotic changes on chest radiograph consistent with prior TB • Patients with organ transplants • Persons who are immunosuppressed for other reasons (e.g., taking the equivalent of >15 mg/day of prednisone for 1 month or longer, taking TNF-alpha antagonists) 	<p>An induration of 10 or more millimeters is considered positive in</p> <ul style="list-style-type: none"> • Recent immigrants (< 5 years) from high-prevalence countries • Injection drug users • Residents and employees of high-risk congregate settings • Mycobacteriology laboratory personnel • Persons with clinical conditions that place them at high risk • Children < 4 years of age • Infants, children, and adolescents exposed to adults in high-risk categories 	<p>An induration of 15 or more millimeters is considered positive in any person, including persons with no known risk factors for TB. However, targeted skin testing programs should only be conducted among high-risk groups.</p>
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Candidates for Treatment of Latent Tuberculosis Infection (LTBI)



- Test only persons at high risk for tuberculosis infection.
- Support adherence to ensure successful treatment completion.
- Do not begin LTBI treatment until active TB disease (pulmonary and extrapulmonary) has been ruled out.

Category of Person Tested	Tuberculin Skin Test (TST) Result (induration)				IGRA positive
	< 5 mm	≥ 5 mm	≥ 10 mm	≥ 15 mm	
Child < 5 years of age and recent close contact ²	Treat	Treat	Treat	Treat	Treat
HIV-infected and recent close contact ²	Treat	Treat	Treat	Treat	Treat
Immunosuppressed and recent close contact ²	Treat	Treat	Treat	Treat	Treat
Recent contact of infectious TB case	Do Not Treat	Treat	Treat	Treat	Treat
HIV-infected	Do Not Treat	Treat	Treat	Treat	Treat
Immunosuppressed or organ transplant recipient	Do Not Treat	Treat	Treat	Treat	Treat
Fibrotic changes on chest x-ray (old inactive TB)	Do Not Treat	Treat	Treat	Treat	Treat
Foreign-born from (or extensive travel to) high-prevalence country ³	Do Not Treat	Do Not Treat	Treat	Treat	Treat
Injection drug user	Do Not Treat	Do Not Treat	Treat	Treat	Treat
Resident/employee of high-risk congregate setting or health care worker ⁴	Do Not Treat	Do Not Treat	Treat	Treat	Treat
Mycobacteria lab personnel ⁴	Do Not Treat	Do Not Treat	Treat	Treat	Treat
High-risk clinical conditions ⁵	Do Not Treat	Do Not Treat	Treat	Treat	Treat
Child < 4 years of age	Do Not Treat	Do Not Treat	Treat	Treat	Treat
Child or adolescent exposed to high-risk adults	Do Not Treat	Do Not Treat	Treat	Treat	Treat
No risk factors (TB screening discouraged)	Do Not Treat	Do Not Treat	Do Not Treat	Treat	Treat
Pregnancy: Candidates for therapy per criteria in table should be treated during pregnancy if either HIV-infected or recently infected.					

TUBERCULIN SKIN TEST

INTRADERMOREAZIONE TUBERCOLINICA SECONDO MANTOUX

SENSIBILITY

False-Negative Reactions

- Cutaneous anergy (*anergy* is the inability to react to skin tests because of a weakened immune system)
- Recent TB infection (within 8-10 weeks of exposure)
- Very old TB infection (many years)
- Very young age (less than 6 months old)
- Recent live-virus vaccination (e.g., measles and smallpox)
- Overwhelming TB disease
- Some viral illnesses (e.g., measles and chicken pox)
- Incorrect method of TST administration
- Incorrect interpretation of reaction

SPECIFICITY

False-Positive Reactions

- Infection with nontuberculosis mycobacteria
- Previous BCG vaccination
- Incorrect method of TST administration
- Incorrect interpretation of reaction
- Incorrect bottle of antigen used



Effetto della vaccinazione con BCG sul TST

INT J TUBERC LUNG DIS 10(11):1192-1204
© 2006 The Union

REVIEW ARTICLE

False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria?

M. Farhat,^{*†} C. Greenaway,^{*‡} M. Pai,^{*§} D. Menzies*

- Analisi di 24 studi con N = 240,243 soggetti
- Quando il BCG è somministrato nell'infanzia, 6% falsi-positivi nei risultati del TST a causa del BCG
- Quando il BCG è somministrato dopo l'infanzia, 40% falsi-positivi a causa del BCG

IGRAs



T-SPOT[®].TB



ANTIGENI:
ESAT-6, CFP-10



QuantiFERON[®]-TB Gold In-Tube

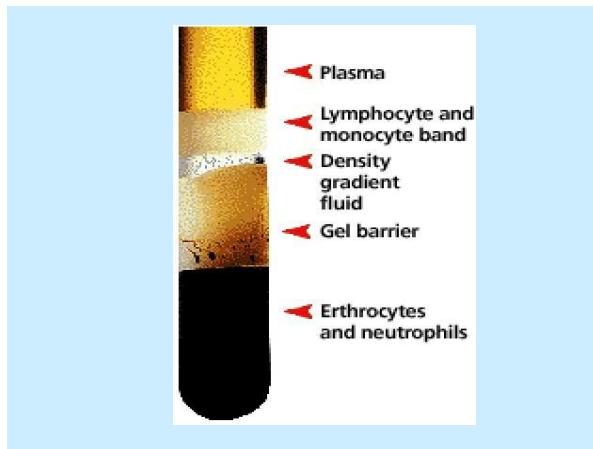


ANTIGENI:
ESAT-6, CFP-10, TB 7.7

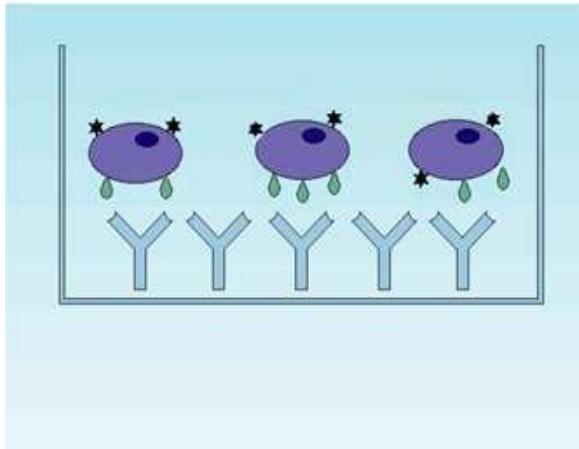
IFN- γ test e Cellule Effettivi

- Le cellule T effettivi producono IFN- γ entro poche ore dalla stimolazione
- Le cellule T memoria devono proliferare e richiedono più di 24 ore prima di una significativa produzione di IFN- γ
- I test IGRA richiedono una incubazione inferiore alle 24 ore
→ L'IFN- γ viene prodotto dalle cellule T effettivi

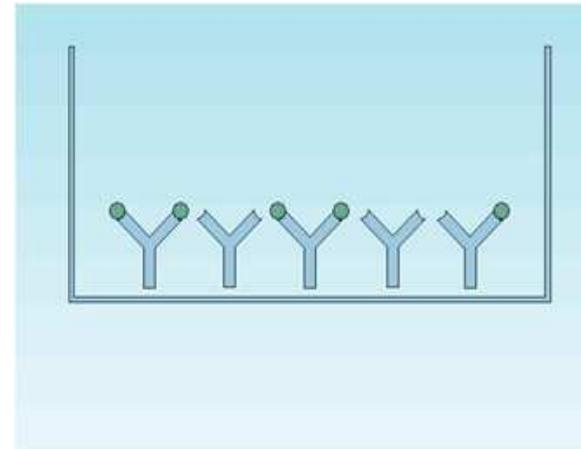
Tecnologia di lavoro



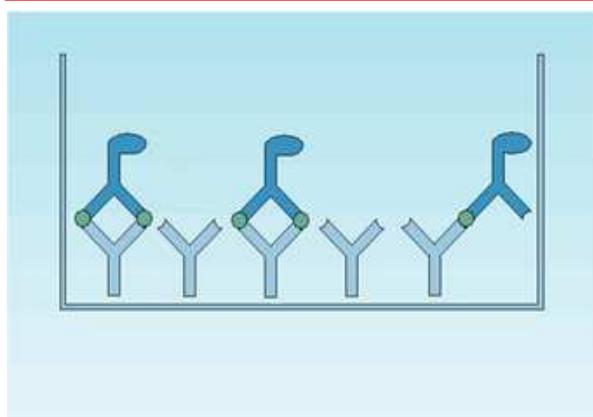
Raccogliere i linfociti usando i tubi vacutainer BD CPT o l'estrazione con Ficoll.



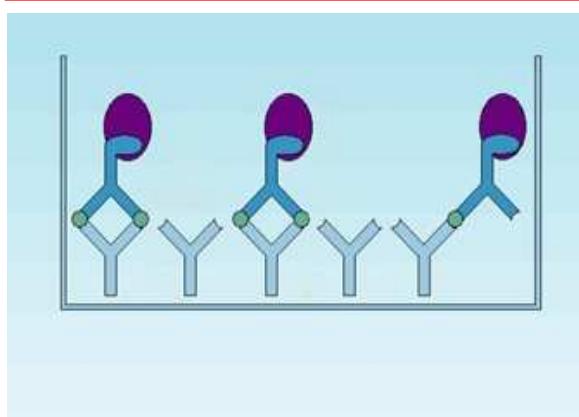
Aggiungere i linfociti e gli antigeni tubercolari ai pozzetti. I linfociti T rilasciano IFN- γ .



L' IFN- γ viene catturato dagli anticorpi.



Incubare, lavare ed aggiungere il coniugato (anticorpi anti IFN- γ).



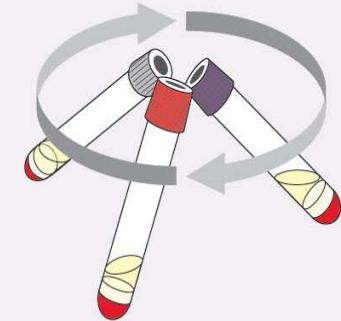
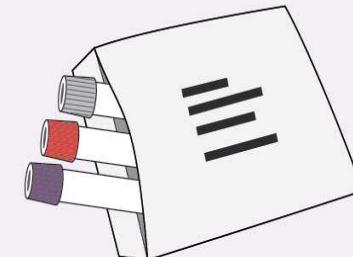
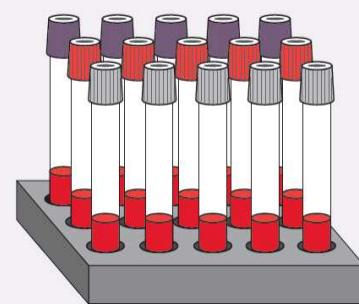
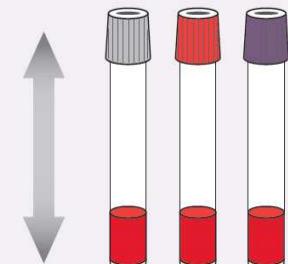
Aggiungere il substrato e contare gli spot colorati in ciascun pozzetto.



Ciascuno spot costituisce un singolo linfocita T che ha rilasciato IFN- γ .

QuantiFERON®-TB Gold In-Tube Passaggi Principali

Parte 1. Incubazione sangue



Dopo il prelievo di sangue capovolgere ripetutamente i tubi per 5 sec.

Appena possibile, ma entro 16 h dal prelievo, incubare i tubi a 37°C per 16-24h

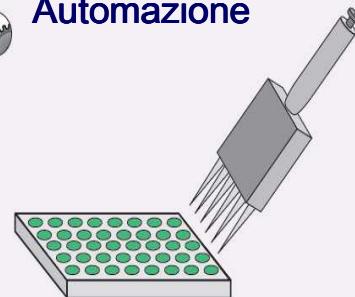
Dopo incubazione i tubi sono stabili per 3 gg a T. ambiente. Possibilità di invio al laboratorio

Centrifugare per 15 min. Conservare il plasma per 8 settimane a 2-8°C, per anni a -20°C

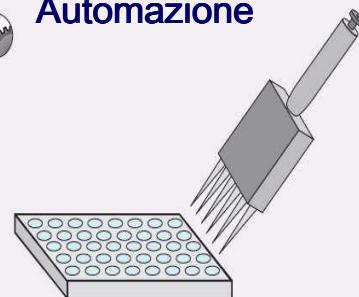
Parte 2. INF- γ ELISA



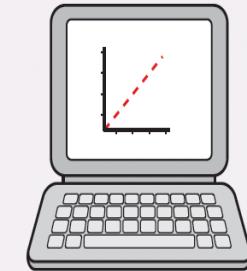
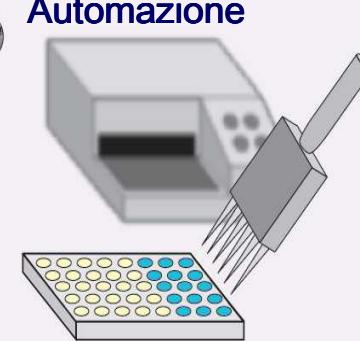
Automazione



Automazione



Automazione



Aggiungere plasma e coniugato ed incubare per 120 min. a T. ambiente

Lavare, aggiungere substrato ed incubare per 30 min. a T. ambiente

Aggiungere la soluzione di stoppaggio e leggere l'assorbanza

Calcolare i risultati usando il software specifico e stampare i referti.

T-SPOT®.TB INTERPRETAZIONE RISULTATI

Numero Spot Controllo Negativo	Numero Spot ESAT-6 E/O CFP10 – Controllo Negativo	Controllo Positivo (PHA)	Risultato
0 - 10	≥ 6	≥ 20 o < 20	Positivo
0 - 10	< 6	≥ 20	Negativo
0 - 10	< 6	< 20	Indeterminato
> 10	≥ 6 o < 6	≥ 20	Indeterminato



0 - 10	≥ 8	≥ 20 o < 20	Positivo
0 - 10	≤ 4	≥ 20	Negativo
0 - 10	≤ 4	< 20	Indeterminato
> 10	≥ 8 o ≤ 4	≥ 20	Indeterminato
0 - 10	5 - 6 - 7	≥ 20	Borderline



FDA Criteria

QuantiFERON®-TB Gold In-Tube

INTERPRETAZIONE RISULTATI

Controllo Negativo UI/ml	Antigeni TB ESAT-6 , CFP10 , TB7.7 - Controllo negativo UI/ml	Controllo Positivo (PHA) UI/ml	Risultato
< 8	≥ 0,35	≥ 0,50 o < 0,50	Positivo
< 8	< 0,35	≥ 0,50	Negativo
< 8	< 0,35	< 0,50	Indeterminato
> 8	≥ 0,35 o < 0,35	≥ 0,50	Indeterminato

Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a meta-analysis.

Diel R. et al. Chest 2010 Apr;137(4):952-68

TST (25 studies)

Pooled Sensitivity = 70%

QFT IT (19 studies)

Pooled Sensitivity = 81%

developed countries (13)

Pooled Sensitivity = 84%

developing countries (6)

Pooled Sensitivity = 74%

T-SPOT. TB (17 studies)

Pooled Sensitivity = 87,5%

QFT IT (5 studies)

Pooled Specificity = 99%

T-SPOT. TB (3 studies)

Pooled Specificity = 86%

TEST IGRA

Sebbene i test IGRA abbiano dimostrato una buona sensibilità, UN RISULTATO NEGATIVO NON ESCLUDE LA POSSIBILITÀ CHE IL SOGGETTO IN ESAME POSSA AVERE UNA LTBI, e non deve mai essere utilizzato da solo per escludere la malattia tubercolare in persone con sintomi o segni compatibili con una TB attiva.

I TEST IGRA POSITIVI NON DISTINGUONO UNA LTBI DA UNA MALATTIA ATTIVA, come la Mantoux.

Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a metaanalysis.

Diel R. et al. Chest 2010 Apr;137(4):952-68

The pooled rate of indeterminate results was:

QFT-IT

2.1%

T-Spot.TB

3.8%

Immunosuppressed hosts

QFT-IT

4.4%

T-Spot.TB

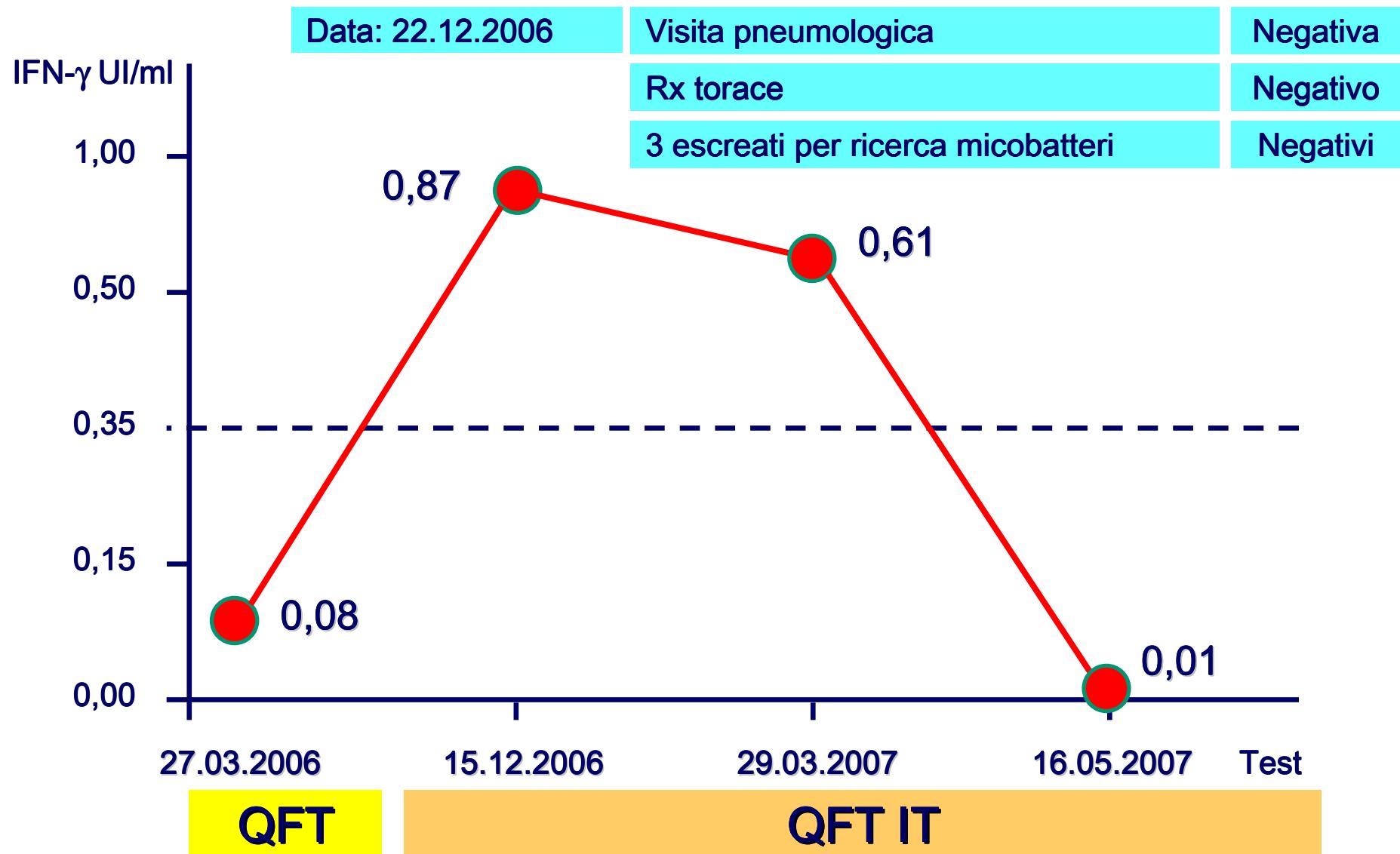
6.1%

There is no doubt that indeterminate results for any of the IGRAs are largely the result of two different factors: a high level of immunosuppression and/or technical error in blood collection and handling or assay performance.

Storie di vita vissuta

Cognome e Nome: Scarparo Claudio

Dirigente Medico: U.O. di Microbiologia



Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review

Zwerling A. et al. Thorax. 2011 Jan 12.

Study	Duration between testing	TST converters, n/N (%)	IGRA converters, n/N (%)	IGRA reverters, n/N (%)
High TB incidence countries				
Pai, 2006 ¹⁵	18 months	6/147 (4.1%)	17/147 (11.6%)	7/38 (18.4%)
Joshi, 2009 ⁵⁸	6 months	–	11/57 (19%)	6/22 (27%)
	6 months (6–12 months)	–	11/52* (21%)	11/27† (40%)
Moderate and low TB incidence countries				
Pollock, 2009 ⁶⁴	1–7 months	–	2/43‡ (4.6%)	–
Zwerling, 2009 ⁵⁹	1 year	0/57 (0%)	4/56 (7.14%)	4/5 (80%)
Yoshiyama, 2009 ⁶⁰	2 and 4 years	–	5/277 (1.8%)	13/32 (41%)
Chee, 2009 ⁶¹	1 year	0/18‡	9/182 (4.9%)	–
Lee, 2009 ⁶²	1 year	16/75 (21.3%)	21/146 (14.4%)	–
Belknap, 2010 ⁶³	6 months	4/1202 (0.3%)	TSPOT 44/1117 (3.9%) QFT-GIT 44/1169 (3.8%)	TSPOT 36/68 (52.9%) QFT-GIT 20/50 (40%)
Costa, 2010 ²⁰	1–2.5 years	98/199 (49.2%)	51/462 (11%)	46/208 (22.1%)
Ringshausen, 2010 ²¹	18 weeks	(baseline only)	3/162 (1.9%)	6/18 (33.3%)

T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India

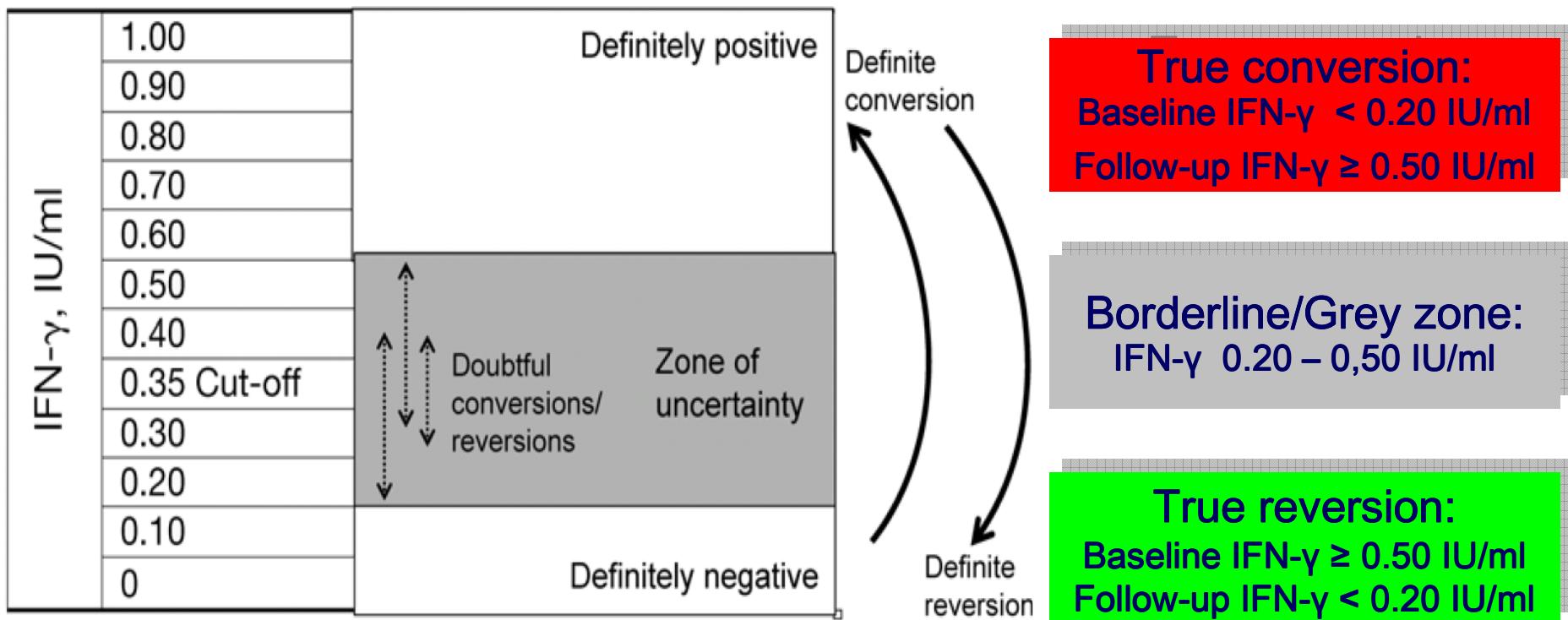
Pai M. et al. Int J Tuberc Lung Dis 2009; 13(1):84-92

QFT conversion

baseline IFN- γ < 0.35 IU/ml and follow-up IFN- γ \geq 0.35 IU/ml
(i.e., a negative to positive change, as recommended by the CDC).

QFT reversion

baseline IFN- γ > 0.35 and follow-up IFN- γ < 0.35 IU/ml.



Within-Subject Variability and Boosting of T-Cell Interferon- γ Responses after Tuberculin Skin Testing

van Zyl-Smit RN, et al. Am J Respir Crit Care Med 2009 Jul 1;180(1):49-58.

TABLE 5. COMPARISON OF QUANTIFERON-TB GOLD IN-TUBE AND T-SPOT.TB VARIABILITY, BORDERLINE ZONES, AND PROPOSED THRESHOLDS FOR CONVERSION

	QuantiFERON-TB Gold In-Tube	T-SPOT.TB
Manufacturer-defined assay cut-point	>0.35 IU/ml	≥ 6 spots*
Within-subject short-term variability	$\pm 80\%$ of IFN- γ response	± 3 spots
Borderline or uncertainty zone	0.2–0.7 IU/ml	4–8 spots (inclusive)
Proposed conversion threshold	Increase from <0.35 to >0.7 IU/ml	Increase from <6 to >9 spots (inclusive)

* The U.S. Food and Drug Administration borderline zone for result interpretation includes values of 5, 6, and 7 spots.

Clinical Application and Limitations of Interferon- γ Release Assays for the Diagnosis of Latent Tuberculosis Infection

Herrera V, et al. Clin Infect Dis 2011;52(8):1031-1037

Conversions and Reversions

In the event that a test is performed for an individual at low risk of LTBI, conversion from a negative to a positive result may represent a false conversion. A reasonable approach is to withhold treatment and repeat testing.

We and others have shown that, in individuals at high risk, results close to the cutoff for QFT-GIT are more likely to revert and convert during repeat testing [46]. Therefore, a high positive result (i.e., >1.0 IU/mL) is more likely to remain positive and, therefore, should be confirmed and treated as if it is still positive. It is not clear why false-positive results occur, but postulated reasons include concomitant illness at the time of testing, laboratory factors, and nonspecific boosting of IFN- γ responses [34, 47].

How should I interpret an interferon gamma release assay result for tuberculosis infection?

Abubakar I., et al. Thorax 2013;68:298-301.

Table 1 Challenges in interpreting IGRA results in various clinical settings

Subgroups considered for IGRA testing by NICE	Uncertainties of IGAs
New entrants from high incidence countries	Which immigrant subgroups to target?
Contacts of a TB outbreak	What is the meaning of reversion?
Healthcare workers	
Adult contacts of active TB cases	
Paediatric contacts of active TB cases*	False negative rates unknown in high-risk populations
Immunocompromised patients*	
Active TB†	False negative rates in 15–25%
Active and latent TB: effect of treatment†	Increased risk of false negative results Unsuitable for treatment monitoring

*NICE recommends testing with both IGRA and the tuberculin skin test.

†NICE does not recommend IGRA testing but highlights potential uses which are the subject of further study.

IGRA, interferon gamma release assay; NICE, National Institute for Health and Clinical Excellence; TB, tuberculosis.



***GRAZIE
PER
L'ATTENZIONE***