NEWMICRO
Network di Microbiologia e Virologia del Nord Est

INCONTRO DI AGGIORNAMENTO:

Nuove tecnologie nella diagnosi rapida delle infezioni del SNC

Trento 12 Ottobre 2012

MENINGOENCEFALITI SUBACUTE E CRONICHE

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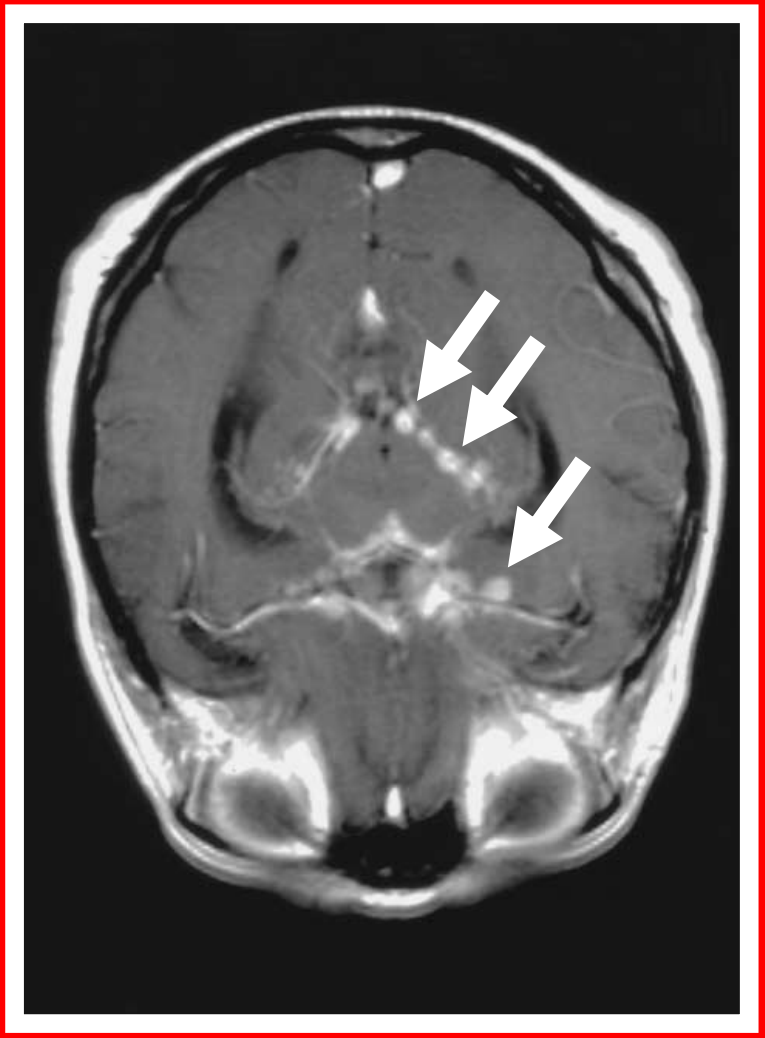
S.O.C. di Microbiologia - Udine

Mycobacterium tuberculosis complex



NEUROTUBERCULOSIS

Affection of central nervous system -

- **Meningeal tuberculosis**
- **Tuberculous encephalopathy**
- **Tuberculoma**
- **Affection of peripheral nervous system - Compressive myelopathy**

Non-compressive

- **Radiculomyelopathy**
- **Polyneuritis**
- **Peripheral neuropathy**
- **Spinal meningeal TB**

European Union Standards for Tuberculosis Care

Migliori GB, et al. Eur Resp J. 2012 Apr;39(4):807-19.

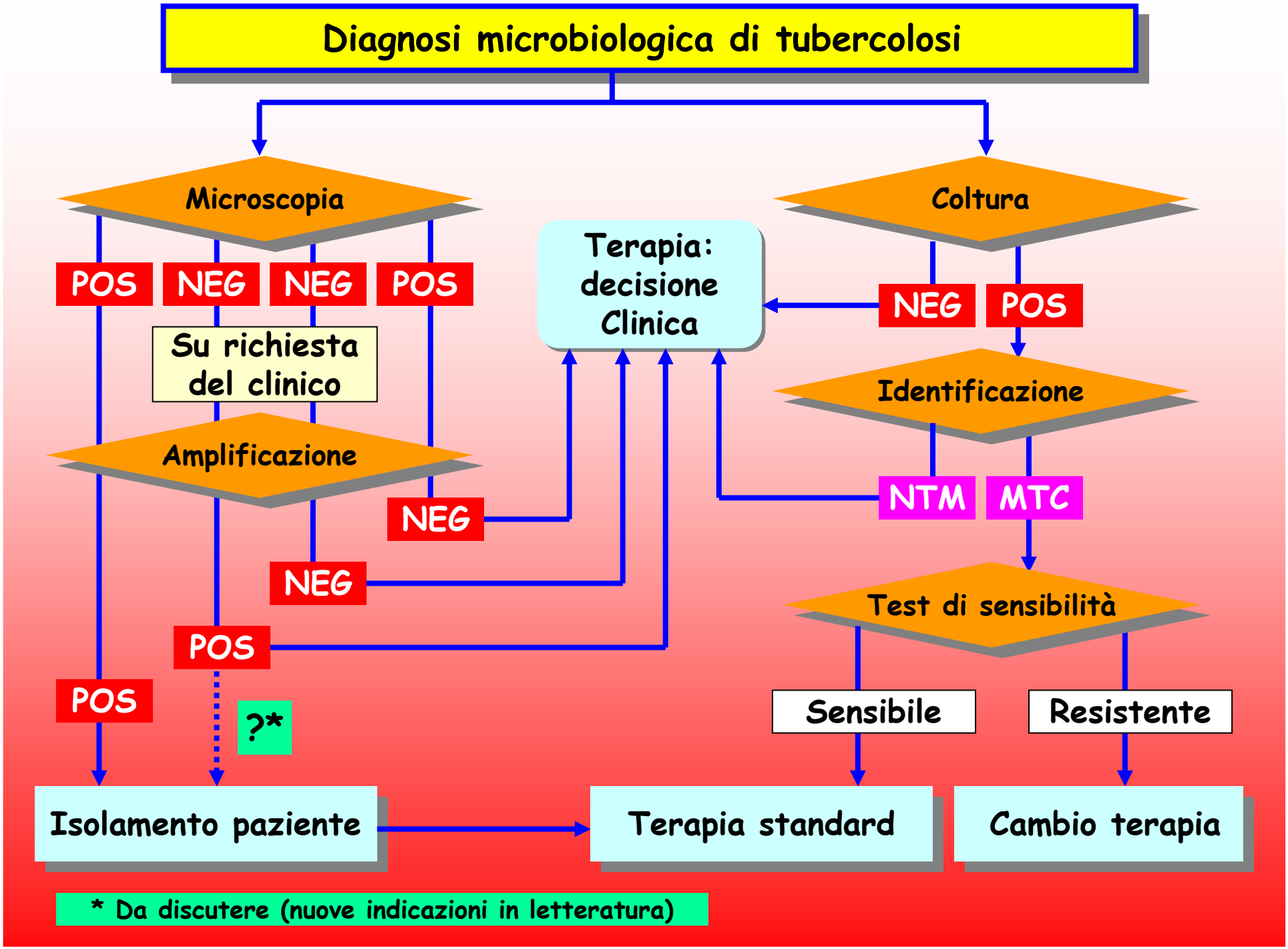
Standard 3 (replaces ISTC 3¶)

For all patients (adults, adolescents and children) suspected of having extrapulmonary TB, appropriate specimens from the suspected sites of involvement should be obtained for microscopy, culture, DST and histopathological examination in a quality-assured laboratory. In countries, settings or populations in which MDR-TB is suspected in a patient, rapid testing for the identification of rifampicin and isoniazid resistance in a quality-assured laboratory could be performed.

Notes

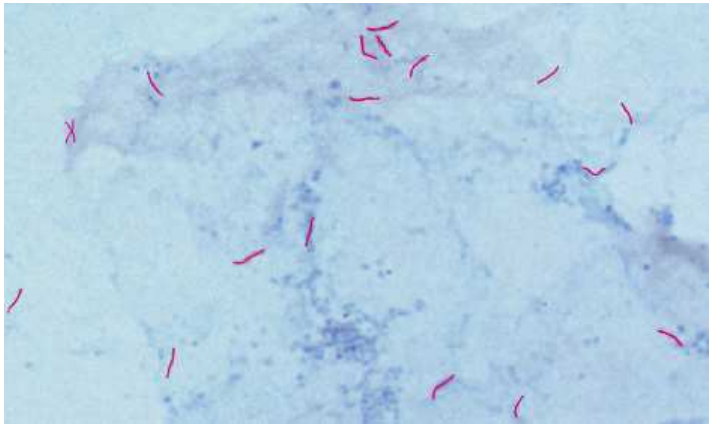
1) This third standard has been accordingly updated to be in line with standards 1 and 2 with regard to essential, standard diagnosis.

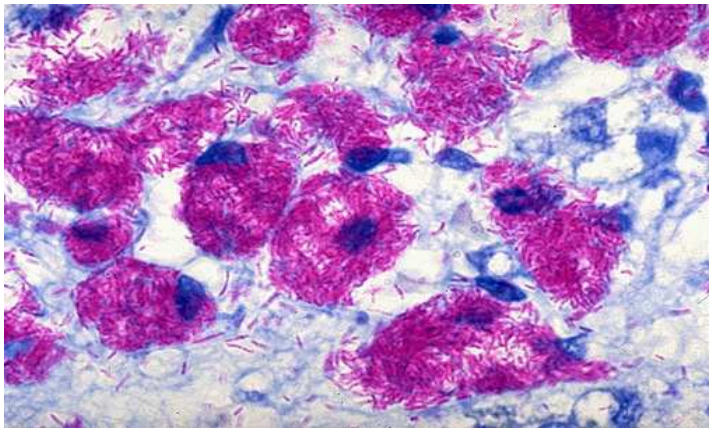
2) It is essential to obtain bacteriological confirmation from extrapulmonary sites in order to confirm diagnosis and consequently provide optimal and effective treatment; this may include the more sensitive molecular test [4].


Diagnosi microbiologica di tubercolosi
Microscopia
Coltura
POS
NEG
NEG
POS
Su richiesta del clinico
Terapia: decisione Clinica
NEG
POS
Identificazione
Amplificazione
NEG
NTM
MTC
NEG
Test di sensibilità
POS
POS
?*
Sensibile
Resistente
Isolamento paziente
Terapia standard
Cambio terapia
* Da discutere (nuove indicazioni in letteratura)

ESAME MICROSCOPICO

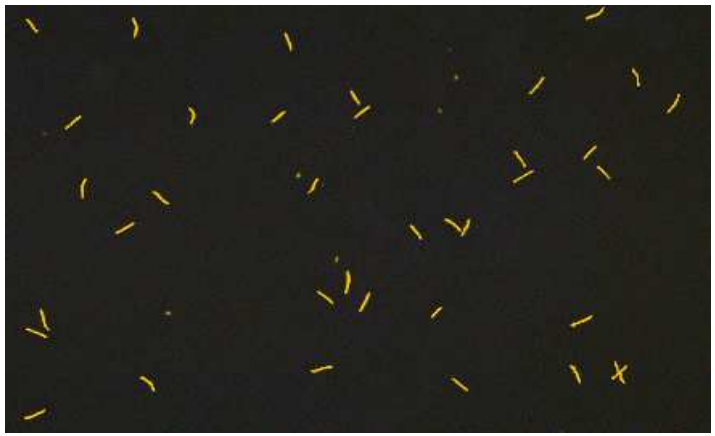
Ziehl-Neelsen

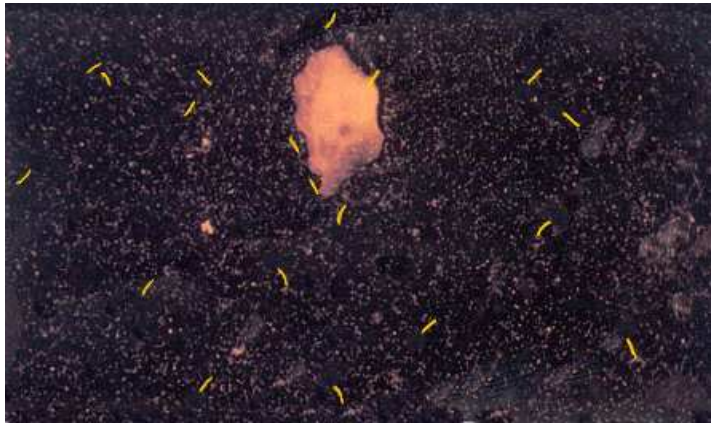




Ob. 100 x - 1 vetrino in 15 minuti

Fluorescenza





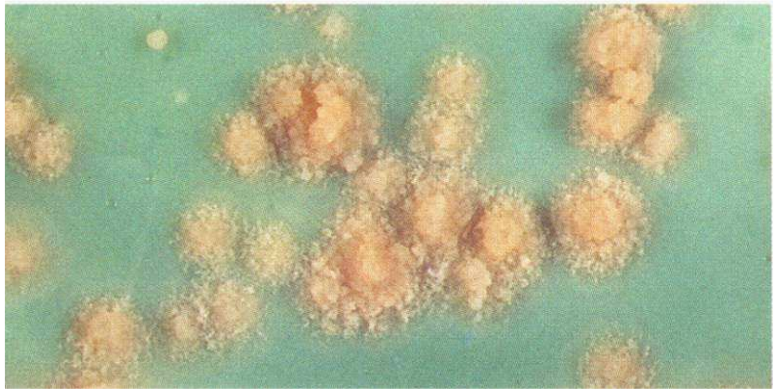
Ob. 20 x - 1 vetrino in 2 minuti

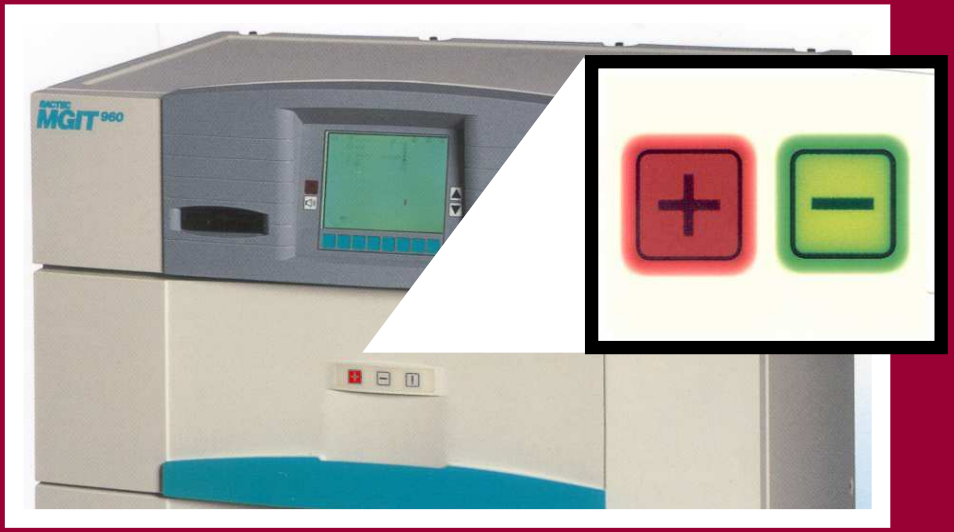
Limite: 5.000 - 10.000 micobatteri/ml

Sensibilità 40 - 60 %

ESAME COLTURALE: TERRENO LIQUIDO + SOLIDO

+



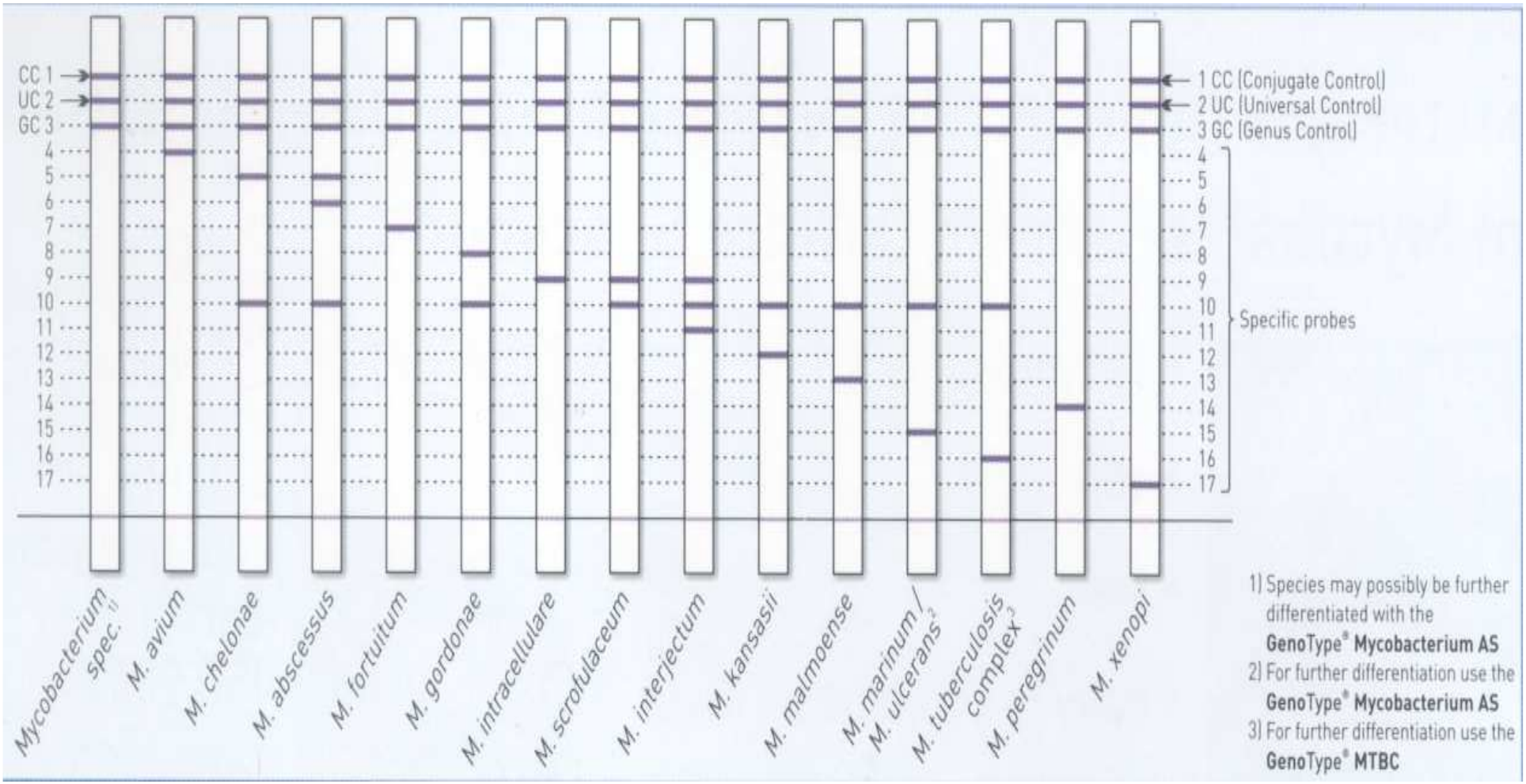


Sensibilità della coltura: 10 - 100 micobatteri/mL.

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



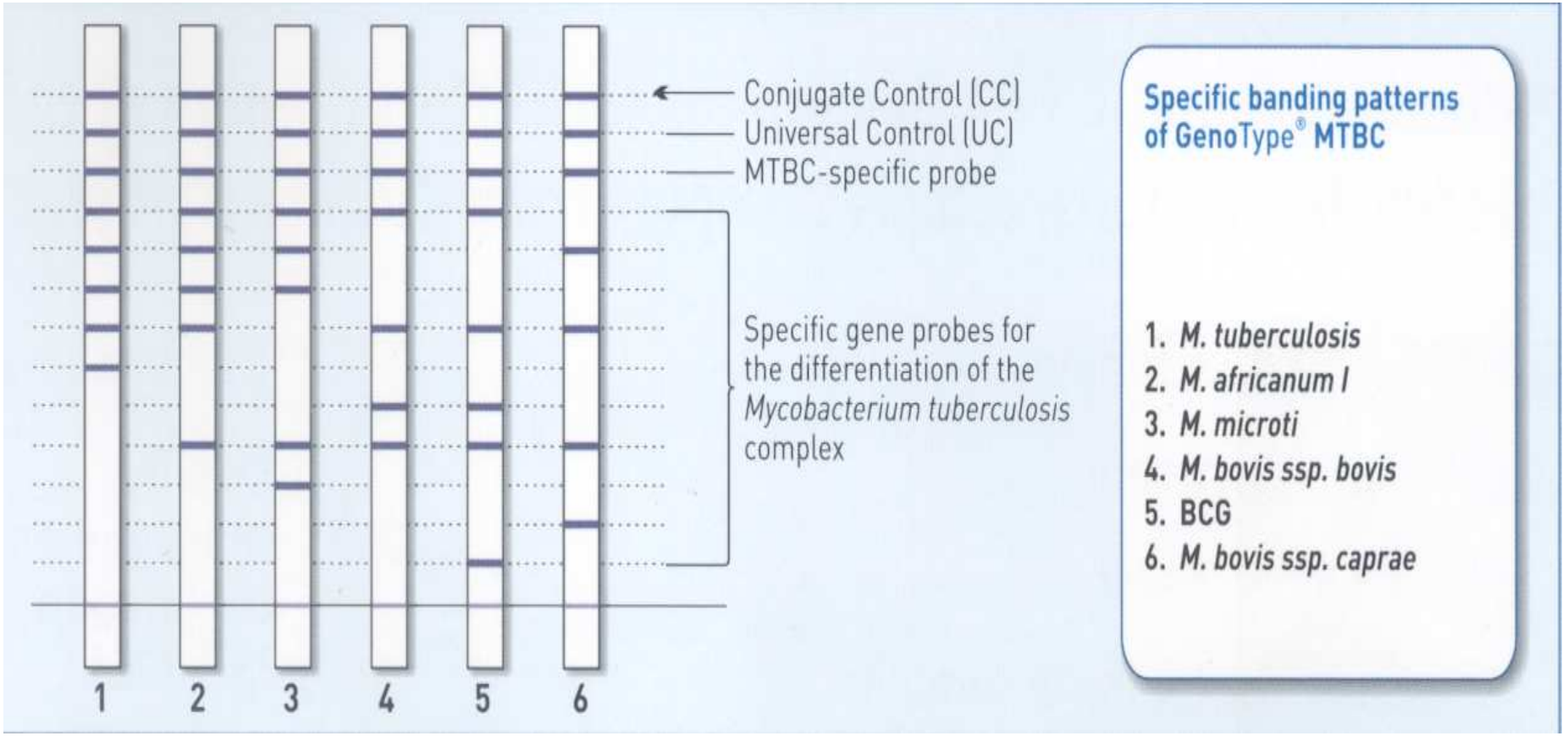
GenoType MTBC (Hain)

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

MTB complex

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

GenoType® MTBC



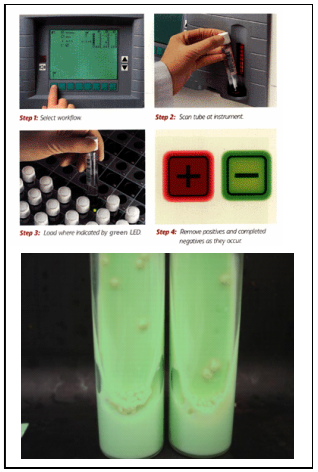
TEST DI SENSIBILITA'

Antimicrobial Susceptibility Testing (AST)

per *M. tuberculosis* complex

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

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, Kanamcina	"Iniettabili"
Polipeptidi	Capreomcina	
Fluorochinoloni	Ciprofloxacina, Ofloxacina, Moxifloxacina	

M. tuberculosis complex: RESISTENZE 2010 IN ITALIA

ISONIAZIDE	10,2%
RIFAMPICINA	3,9%
ETAMBUTOLO	2,8%
STREPTOMICINA	9%
MDR	3,4%
XDR	Rari

XDR-TB

Countries reporting XDR-TB as of March 2010

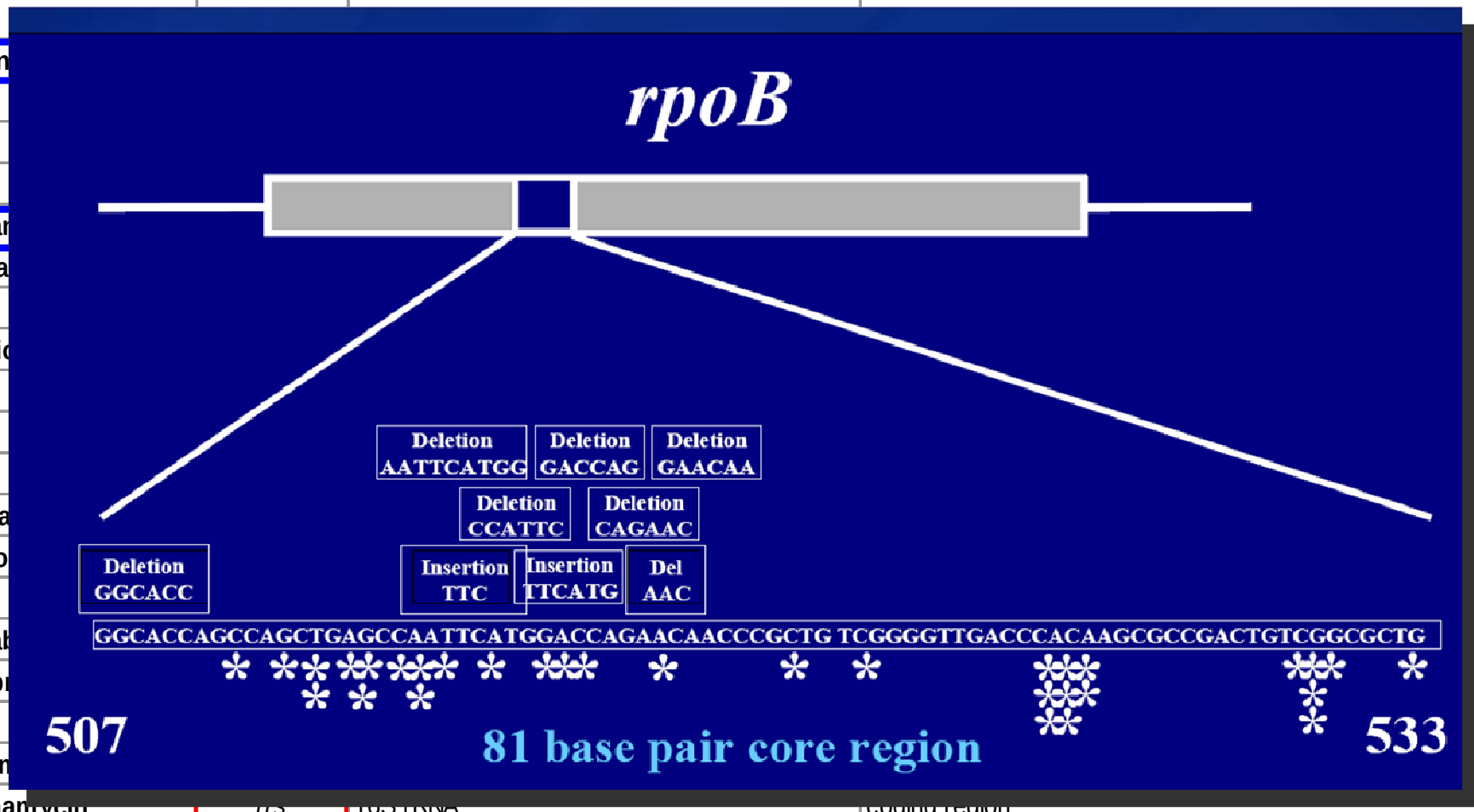
XDR-TB Findings:

- 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

Geni coinvolti nella resistanza ai farmaci anti-tuberculari

Drug	Gene	Gene product	Mutations
Streptomycin	*rpsL*	12S ribosomal protein	Coding region (60%)
Ison			
Rifar			
Etha			
Ethic			
Pyra			
Fluo			
Rifab			
Capr			
Vion			
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"

- Key-drug per il regime di trattamento
- Le mutazioni sono concentrate in un hot-spot del gene *rpoB*

Candidato ottimale per la diagnosi molecolare

Metodi:

-"Home made"

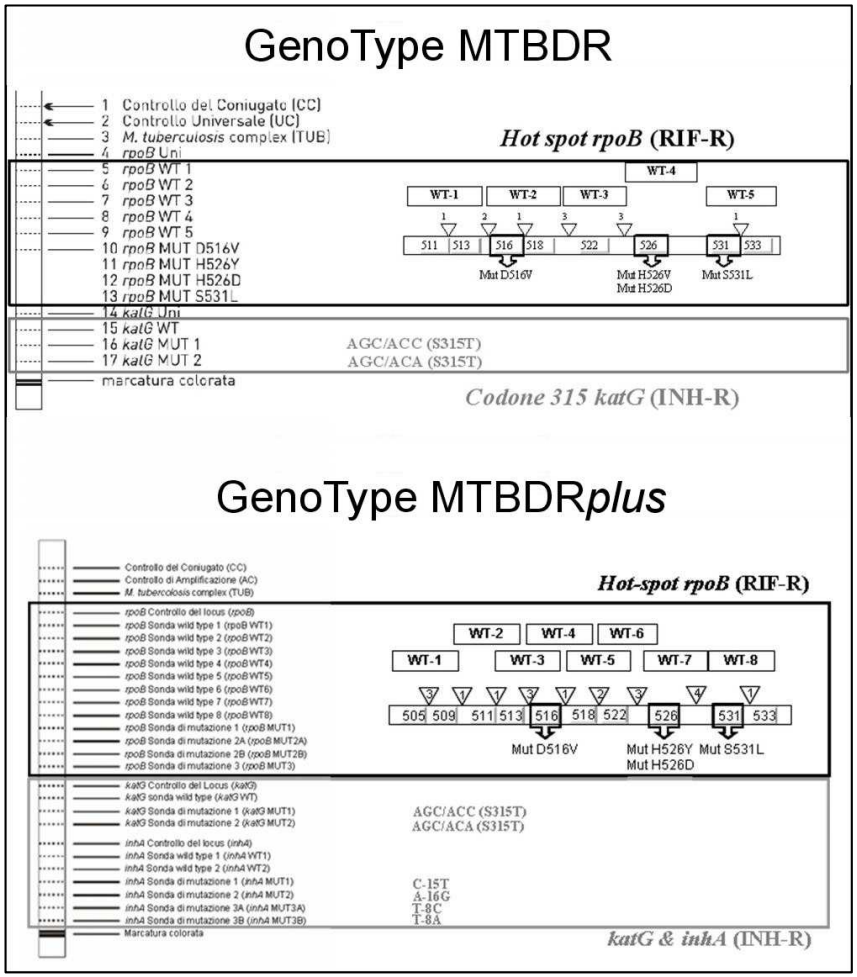
- Commerciali

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*) correlano 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

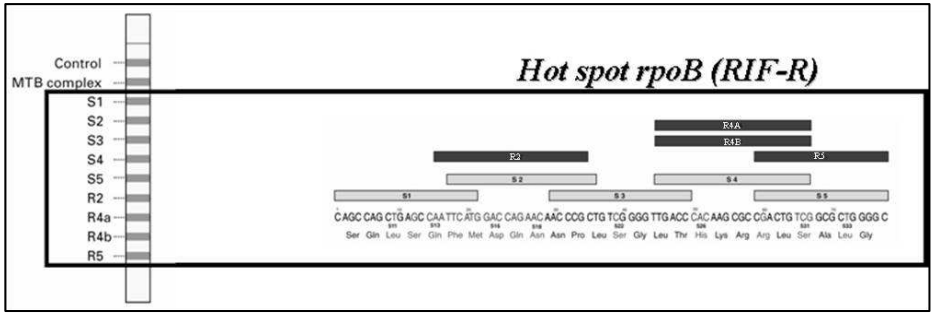
Test commerciali per la farmacoresistenza

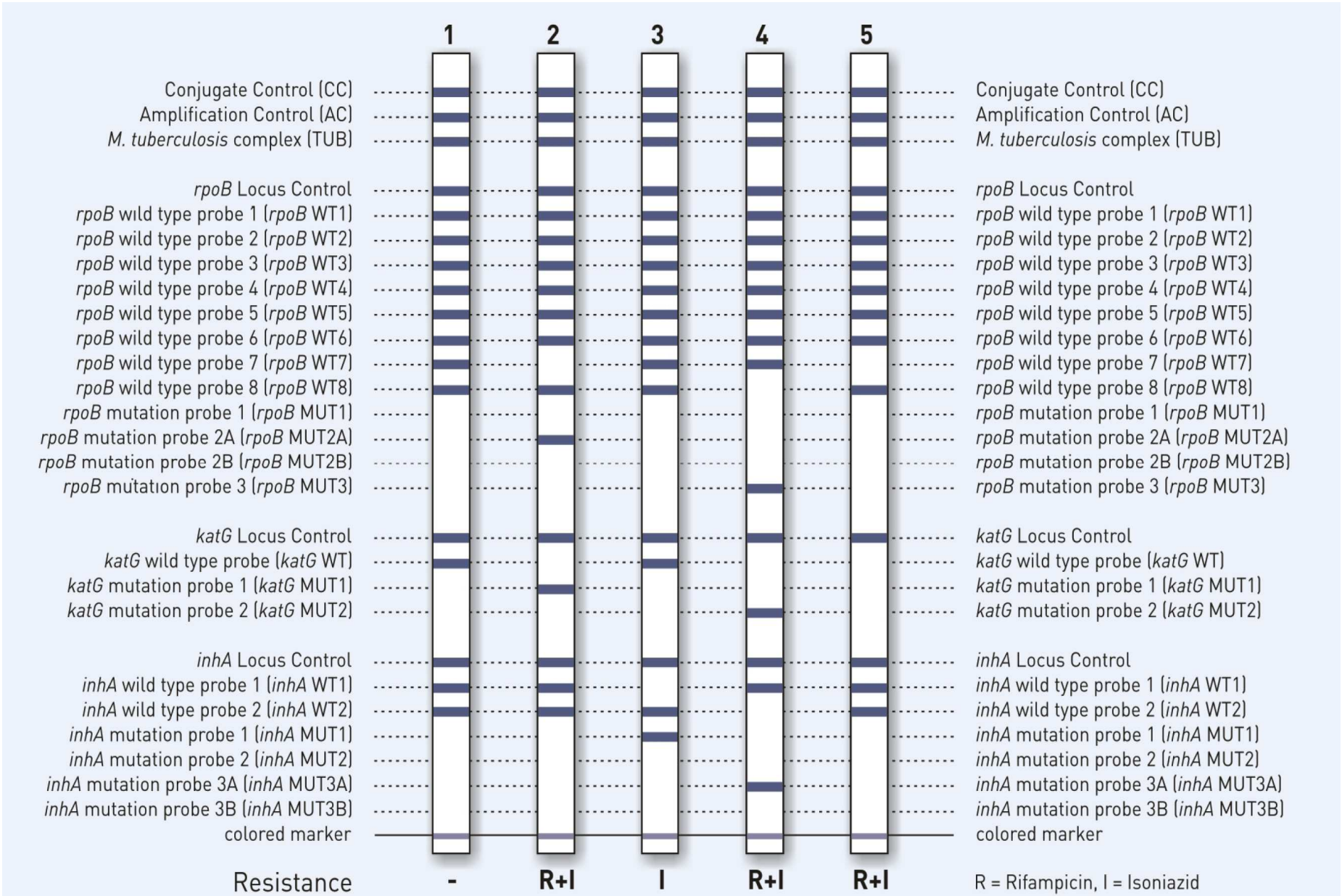
Hain Lifescience



Innogenetics

INNO-LiPA-Rif.TB





Reaction zones of GenoType® MTBDRplus (examples)

GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis

Ling DI, et al. Eur Respir J 2008; 32: 1165–1174

TABLE 5 Pooled summary estimates for rifampicin resistance

Subgroup	Pooled sensitivity	Pooled specificity	Pooled LR+	Pooled LR-	Pooled DOR
All rifampicin studies[#]	98.1 (95.9–99.1)	98.7 (97.3–99.4)	78.0 (36.3–168.0)	0.02 (0.01–0.04)	4010.1 (1205.9–13335.2)
Only MTBDRplus assays[¶]	98.4 (95.1–99.5)	98.9 (96.8–99.7)	95.3 (30.7–296.0)	0.02 (0.005–0.05)	6150.7 (1061.8–35628.9)
Only clinical specimens[+]	98.6 (95.5–99.6)	98.5 (96.9–99.3)	66.3 (31.9–138.0)	0.01 (0.004–0.04)	4659.3 (1064.6–20391.3)

TABLE 7 Pooled summary estimates for isoniazid resistance

Subgroup	Pooled sensitivity	Pooled specificity	Pooled LR+	Pooled LR-	Pooled DOR
All isoniazid studies[#]	84.3 (76.6–89.8)	99.5 (97.5–99.9)	190.6 (33.4–1086.3)	0.16 (0.10–0.24)	1210.8 (175.3–8361.5)
Only MTBDRplus assays[¶]	88.7 (82.4–92.8)	99.2 (95.4–99.8)	112.6 (18.7–677.7)	0.11 (0.07–0.18)	986.8 (133.6–7285.9)
Only clinical specimens[+]	84.5 (72.1–92.0)	99.2 (96.4–99.8)	110.1 (22.3–542.3)	0.15 (0.08–0.29)	706.6 (97.7–5110.8)

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.

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

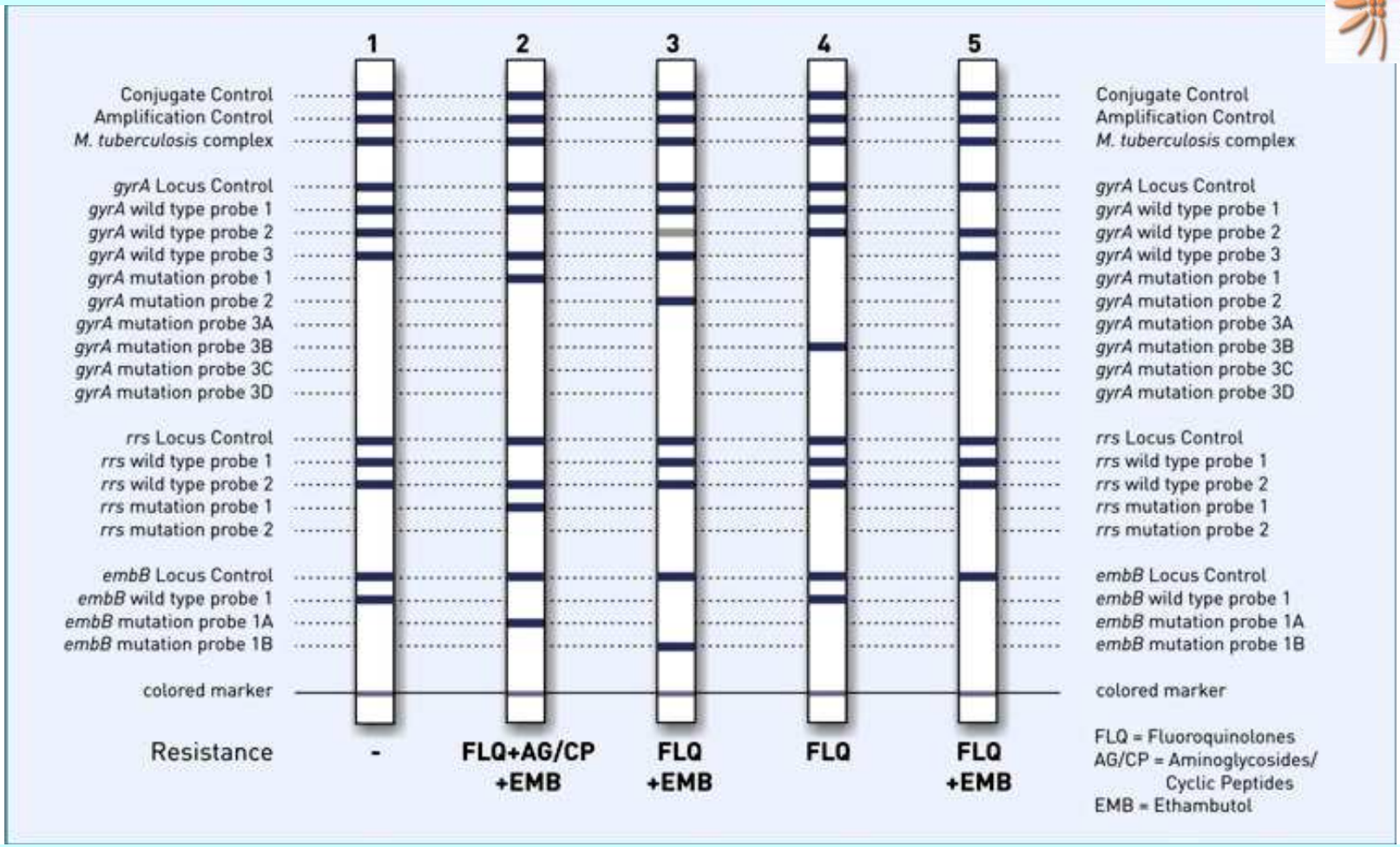
Migliori GB, et al. Eur Resp J. 2008 Jun;31(6):1155-9

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.

GenoType® MTBDR*sl*: identification of the *M. tuberculosis* complex and its resistance to fluoroquinolones, capreomycin, viomycin/kanamycin, amikacin and/or ethambutol

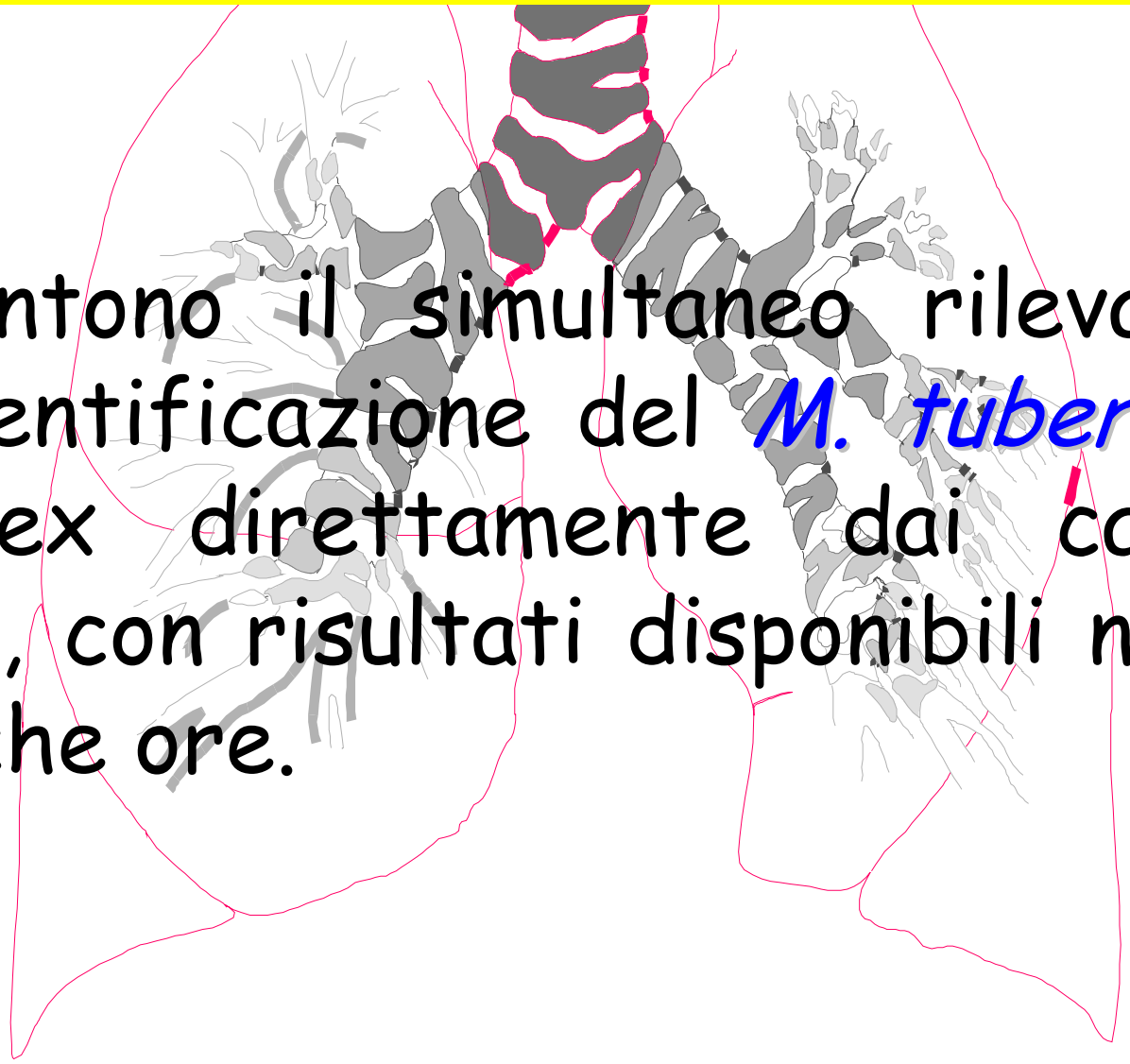


Fluoroquinolones: most significant mutations of *gyrA* gene
Aminoglycosides/Ciclic peptides: most significant mutations of 16s rRNA gene (*rrs*)
Ethambutol: most significant mutations of *emb/B* gene

Molecular DST: conclusions

- *Usefulness of molecular techniques in DST is still limited by the lacks of knowledge of all molecular mechanisms of resistance.*
- *Negative results from genotypic tests **do not exclude** a resistant phenotype*
- *Although these assays cannot replace conventional DST, **the high sensitivity and specificity for RIF-R and INH-R can facilitate the early diagnosis and treatment of MDR-TB, particularly for patients with a history of prior TB treatment.***
- ***Sensitivity of commercial tests could be influenced by geographic regions***
- *Identification of mutations by molecular DST allows to predict cross-resistances among drugs (e.g. aminoglycosids, cyclic peptides).*

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**

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

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

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

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.

Performance of a commercial nucleic acid amplification test with extrapulmonary specimens for the diagnosis of tuberculosis

Piersimoni C, et al. Eur J Clin Microbiol Infect Dis 2012 31:287–293

Table 6 Sensitivities, specificities, and predictive values of smear-negative, extrapulmonary specimens collected from patients with a conclusive diagnosis of tuberculosis (TB)

Performance measure	CSF samples	Sterile body fluids	Biopsy samples	Fine-needle aspirates	Pus samples	Gastric aspirates	Urine	All specimens
Sensitivity (%)	87.5	62.5	90.9	71.4	76.9	93.7	100	81.0
Specificity (%)	100	99.2	90.7	98.6	100	100	100	99.0
PPV (%)	100	83.3	71.4	83.3	100	100	100	90.6
NPV (%)	98.7	97.7	97.5	97.3	96.0	98.4	100	97.8
No. of specimens	83	136	56	237	175	84	145	918

Performance of a commercial nucleic acid amplification test with extrapulmonary specimens for the diagnosis of tuberculosis

Piersimoni C, et al. Eur J Clin Microbiol Infect Dis 2012 31:287–293

Category of specimens	Cerebrospinal fluid samples	
	No. of specimens	DTB-positive
Smear-positive and MTB culture-positive	–	–
Smear-negative and MTB culture-positive	3	2
NTM culture-positive	–	–
Smear- and culture-negative. Collected from patients clinically suspected of having active TB	80[ø]	5(ø)
All categories	83[ø]	7

[ø] Specimens showing inhibition of IAC

(ø) Discrepant specimens conclusively resolved as true-positives

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
Cavitary 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	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)
Cavitary 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

Comparison of DNA extraction protocols for *Mycobacterium Tuberculosis* in diagnosis of tuberculous meningitis by real-time polymerase chain reaction

Thakur R, et al. J Glob Infect Dis. 2011 Oct;3(4):353-6.

Protocols	Shock treatment	Treatment with lysozyme	Chemical lysis	DNA purification	DNA precipitation
QIAGEN Protocol QIAmp spin pr. (M)	+	-	Proteinase K and guanidine HCl	Silica membrane	Tris HCl and EDTA
AMPLICOR Protocol (M)	-	+	NaOH, EDTA and sodium azide	Tris HCl buffer, MgCl and Sodium azide	
MagNA PURE Protocol (A)	-	-	Proteinase K and chaotropic salts	Magnetic glass particles	Low salt elution buffer
MagNA PURE Protocol (M + A)	+	+	Proteinase K and chaotropic salts	Magnetic glass particles	Low salt elution buffer

Extraction Protocols	Samples											
	Run 1				Run 2				Run 3			
	10^3	10^2	$10^{1.5}$	10	10^3	10^2	$10^{1.5}$	10	10^3	10^2	$10^{1.5}$	10
QIAGEN Protocol QIAmp spin pr. (M)	+	-	-	-	+	-	-	-	+	-	-	-
AMPLICOR Protocol (M)	+	-	-	-	+	-	-	-	+	-	-	-
MagNA PURE Protocol (A)	+	-	-	-	+	-	-	-	+	-	-	-
MagNA PURE Protocol (M + A)	+	+	-	-	+	+	-	-	+	+	-	-

IGRAs

Oxford Immunotec

T-SPOT®.*TB*

ANTIGENI:
ESAT-6, CFP-10

cellestis

QuantiFERON®-TB Gold In-Tube

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

Comparative Performance of TST Test, QFT-IT Assay, and T-Spot.TB Test in Contact Investigations for Tuberculosis.

Diel R, et al. *Chest* 2008 Apr;135(4):1010-8.

A recent meta-analysis comparing the two IGRAs suggested QFT was more specific than T-Spot, but less sensitive for detecting active TB[1]. Our results are in discord with this analysis as we found more contacts positive by QFT than T-Spot.

However, we were evaluating both tests performance in people at risk of having LTBI, the primary target for IGRAs, rather than those with active TB reported in the meta-analysis.

Sensitivity of IGRAs has routinely been estimated in patients with active TB, as there is no gold standard for LTBI, but active TB patients have been reported to have reduced IFN-γ responses to TB antigens[2]. This, along with our findings, suggests that IGRA responses of active TB patients may not be representative of responses from people with LTBI.

[1]Pai M, et al. Systematic review: T-Cell-based assays for the diagnosis of latent tuberculosis infection: An update. *Ann Intern Med* 2008; 149:177–184.

[2]Hirsch CS, et al. Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis* 1999; 180:2069-2073.

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%**

Usefulness of the whole-blood interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis

Song KH, et al. Diagn Microbiol Infect Dis 2009 Feb;63(2):182-7

QFT-G:

48 casi di Tubercolosi Extrapolmonare (EP TB) confermati (110 totali)

Sensibilità 69%
Specificità 87%

Non può essere utilizzato come unico criterio per la diagnosi di EP TB

British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children

Thwaites G., et al. J Infect. 2009 Sep;59(3):167-87

Interferon-gamma release assays (IGRA)

Recommendation

CSF adenosine deaminase activity is not recommended as a routine diagnostic test for CNS tuberculosis (B,II). The tuberculin skin test and IGRAs (using peripheral blood) may provide indication of previous tuberculosis infection and are probably most useful in young children, but results need to be interpreted cautiously as neither is sufficiently sensitive nor specific to diagnose active disease. Currently, IGRAs are only licensed for the diagnosis of latent tuberculosis and cannot be recommended for the diagnosis of active CNS disease (B,II).

confirmed TBM had no detectable *M. tuberculosis*-specific interferon-gamma producing lymphocytes in peripheral blood at presentation.[78]

Rapid Diagnosis of Tuberculous Meningitis by T Cell–Based Assays on Peripheral Blood and Cerebrospinal Fluid Mononuclear Cells

Kim SH., et al. Clin Infect Dis. 2010 May 15;50(10):1349-58.

Table 5. Diagnostic Accuracy of Diagnosis of Tuberculosis (TB) Meningitis in 86 Patients with Suspected TB Meningitis

Conclusion. ELISPOT assays using peripheral mononuclear cells and CSF mononuclear cells are useful adjuncts to the current tests for diagnosing TBM, particularly when used in combination with the assessment of adenosine deaminase level in CSF.

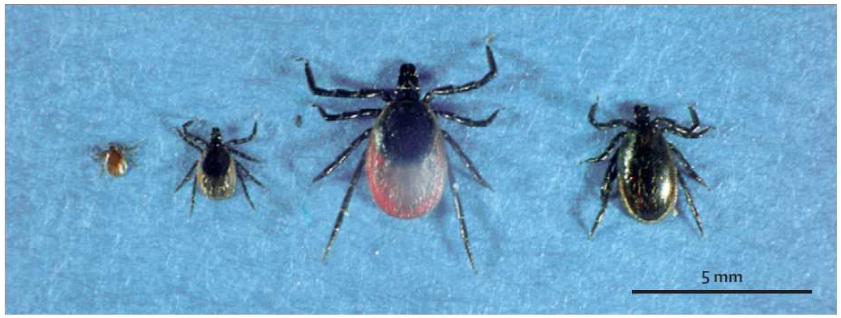
Tuberculin skin test result ≥10 mm	7/31	23 (10–41)	39/45	87 (73–95)	54 (25–81)	62 (49–74)	1.69 (0.63–4.56)	0.90 (0.72–1.12)
PBMC ELISPOT ≥6 spots	22/31	71 (51–86)	30/53	57 (42–70)	49 (34–64)	77 (61–89)	1.64 (1.12–2.39)	0.51 (0.28–0.93)
CSF-MC ELISPOT ≥6 spots	13/22	59 (36–79	25/28	89 (72–98)	81 (54–96)	74 (56–87)	5.52 (1.79–16.98)	0.46 (0.27–0.77)
CSF ADA level >5.8 IU/L	23/26	89 (69–98)	37/51	73 (58–84)	62 (45–78)	93 (79–98)	3.22 (2.02–5.14)	0.16 (0.05–0.47)
CSF/serum glucose ratio ≤0.44	20/27	74 (54–89)	35/51	69 (54–81)	56 (38–72)	83 (69–93)	2.36 (1.49–3.75)	0.38 (0.19–0.73)
PBMC ELISPOT or CSF ADA > 5.8 IU/L	29/31	94 (79–99)	25/55	46 (32–59)	49 (36–63)	93 (76–99)	1.72 (1.32–2.22)	0.14 (0.03–0.55)

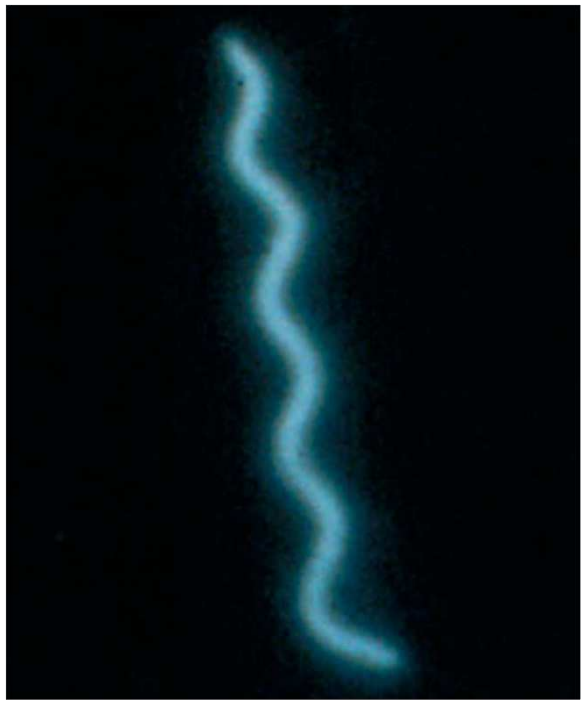
NOTE. ADA, adenosine deaminase; CI, confidence interval; CSF, cerebrospinal fluid; CSF-MC, cerebrospinal fluid-mononuclear cell; ELISPOT, enzyme-linked immunosorbent spot; NPV, negative predictive value; PBMC, peripheral blood mononuclear cell; PPV, positive predictive value; TB, tuberculosis.

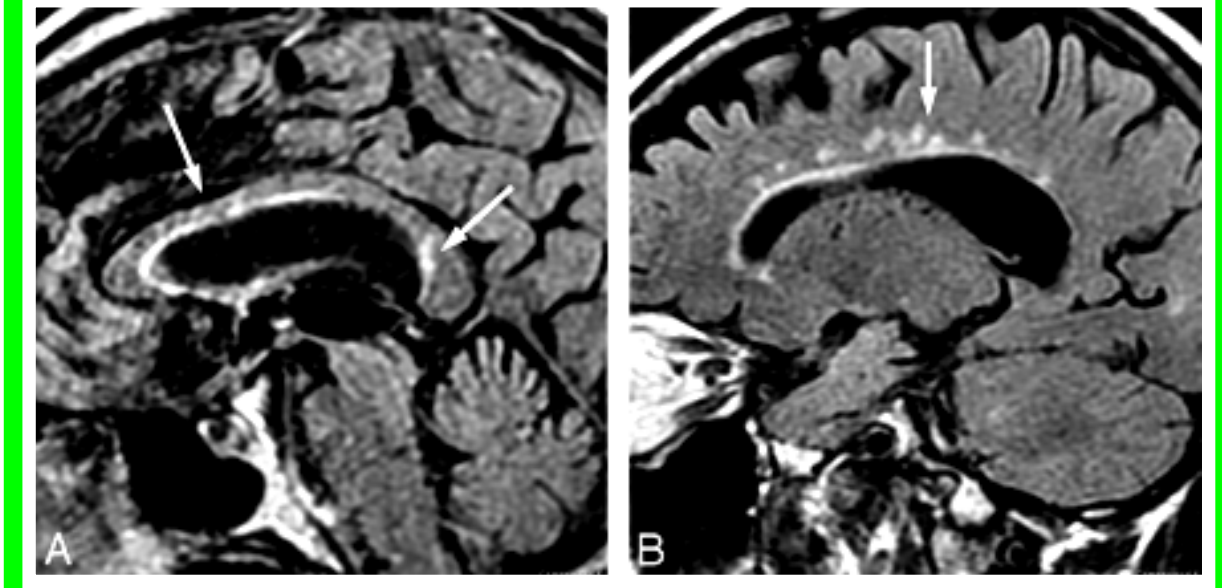
[a] Sensitivity is determined by dividing the no of patients with a positive test result by the no of patients tested.

[b] Specificity is determined by dividing the no of patients with a negative test result by the no of patients tested.

Borrelia burgdorferi “sensu lato”







Lyme borreliosis

Stanek G, et al. Lancet 2012; 379: 461–73

	Primary diagnostic testing	Supporting diagnostic testing	Supporting clinical findings
Erythema migrans			
Expanding red or bluish-red patch (≥5 cm in diameter),* with or without central clearing Advancing edge is typically distinct, often intensely coloured, and not noticeably raised	Diagnosis on the basis of history and visual inspection of the skin lesion Laboratory testing not needed or recommended If lesion is atypical, then acute-phase and convalescent-phase serological testing† are recommended because of insensitivity of acute phase testing‡	Culture or PCR of a skin biopsy specimen useful in research studies, but not needed for routine clinical practice	Tick bite at site; regional lymphadenopathy in North American patients
Lyme neuroborreliosis			
In adults, mainly meningo-radiculitis, meningitis, and peripheral facial palsy; rarely encephalitis, myelitis; very rarely cerebral vasculitis	Pleocytosis and demonstration of synthesis of intrathecal antibodies to *Borrelia burgdorferi* sensu lato§	Detection of Lyme borrelia by culture or PCR of cerebrospinal fluid Intrathecal synthesis of total IgM, IgG, or IgA	Recent or concomitant erythema migrans
In children, mainly meningitis and peripheral facial palsy	Serological testing† usually positive at time of presentation; if negative, test convalescent phase sera (2–6 weeks later)		

Table 1: Manifestations, brief clinical case definitions, and recommended diagnostic approach for the diagnosis of Lyme borreliosis in routine clinical practice

§In early cases, intrathecally produced specific antibodies might still be absent.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis

Mygland A, et al. Eur J Neurol. 2010 Jan;17(1):8-16, e1-4

Table 1 Classification of Lyme neuroborreliosis (LNB)

Early LNB
Neurological symptoms for < 6 months
With manifestations confined to PNS (cranial nerves, spinal roots or peripheral nerves) (Bannwarth syndrome)
With CNS manifestations
Late LNB
Neurological symptoms for more than 6 months
With PNS manifestations
With CNS manifestations

PNS, peripheral nervous system.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis

Mygland A, et al. Eur J Neurol. 2010 Jan;17(1):8-16, e1-4

Recommendations

Choice of laboratory methods

1. Investigation of CSF/serum pair for *Bb*-specific antibodies, intrathecal antibody production and signs of CSF inflammation is obligatory for laboratory diagnosis of LNB (level B).

2. Culture and PCR may be corroborative in very early LNB (GPP).

3. At present, no further methods are recommendable.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis

Mygland A, et al. Eur J Neurol. 2010 Jan;17(1):8-16, e1-4

Table 2 Suggested case definitions for Lyme neuroborreliosis (LNB)

Definite neuroborreliosis[a]	Possible neuroborreliosis[b]
All three criteria fulfilled	Two criteria fulfilled
Neurological symptoms suggestive of LNB without other obvious reasons	
Cerebrospinal fluid pleocytosis	
Intrathecal *Bb* antibody production	

[a]These criteria apply to all subclasses of LNB except for late LNB with polyneuropathy where the following should be fulfilled for definite diagnosis: (I) peripheral neuropathy (II) acrodermatitis chronica atrophicans (III) *Bb*-specific antibodies in serum.

[b]If criteria III is lacking; after a duration of 6 weeks, there have to be found *Bb*-specific IgG antibodies in the serum.

Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots

Ang CW, et al. Eur J Clin Microbiol Infect Dis 2011 30:1027–1032

Table 1 Performance of eight enzyme-linked immunosorbent assay (ELISAs) in the three patient groups

ELISA manufacturer	Antigen used for ELISA	Number of positive samples (%)			Total number of tested samples
		Patients suspected for *Borrelia* infection	Cross-reactivity controls	Healthy controls	
Diacheck/Moran	Whole-cell	20/59 (34%)	2/16 (13%)	1/14 (7%)	89
VIDAS	Whole-cell	31/59 (53%)	4/16 (25%)	1/14 (7%)	89
Virion/Serion	Whole-cell	24/59 (41%)	1/16 (6%)	0/14	89
Enzygnost	Whole-cell+VlsE	23/59 (39%)	0/16	0/14	89
Euroimmun	Whole-cell+VlsE	29/59 (49%)	3/16 (19%)	0/14	89
Virotech	Whole-cell+VlsE	35/59 (59%)	6/16 (38%)	0/14	89
Immunetics	Recombinant	22/59 (37%)	0/16	0/14	89
Mikrogen	Recombinant	24/59 (41%)	3/16 (19%)	0/14	89

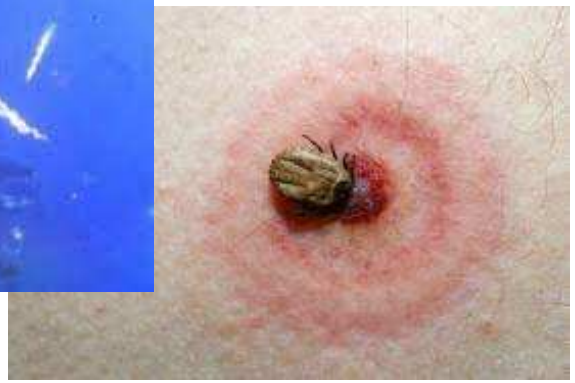
Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots

Ang CW, et al. Eur J Clin Microbiol Infect Dis 2011 30:1027–1032

Table 4 Fractions of blot-confirmed samples for 40 ELISA–immunoblot combinations

ELISA manufacturer	Antigen used for ELISA	Number of positive samples in ELISA/total number of samples	Blot				
			Whole-cell	Whole-cell+VlsE	Recombinant		
			Home-made	Virablot	Euroimmun	Mikrogen	Virotech
Diacheck/Moran	Whole-cell	12/31	11/12 (92%)	9/12 (75%)	11/12 (92%)	12/12 (100%)	9/12 (75%)
VIDAS	Whole-cell	19/31	11/19 (58%)	12/19 (63%)	13/19 (68%)	14/19 (74%)	10/19 (53%)
Virion/Serion	Whole-cell	15/31	11/15 (73%)	11/15 (73%)	13/15 (87%)	12/15 (80%)	9/15 (60%)
Enzygnost	Whole-cell+VlsE	12/31	11/12 (92%)	10/12 (83%)	10/12 (83%)	12/12 (100%)	10/12 (83%)
Euroimmun	Whole-cell+VlsE	14/31	11/14 (79%)	11/14 (79%)	12/14 (86%)	12/14 (86%)	9/14 (64%)
Virotech	Whole-cell+VlsE	17/31	11/17 (65%)	11/17 (65%)	13/17 (77%)	13/17 (77%)	9/17 (53%)
Immunetics	Recombinant	13/31	11/13 (85%)	10/13 (77%)	10/13 (77%)	13/13 (100%)	10/13 (77%)
Mikrogen	Recombinant	13/31	11/13 (85%)	9/13 (69%)	11/13 (85%)	12/13 (92%)	9/13 (69%)

IDEIA™ Lyme Neuroborreliosis



Saggio immunoenzimatico per la determinazione di **anticorpi IgG e IgM intratecali umani contro *Borrelia burgdorferi* sensu lato**

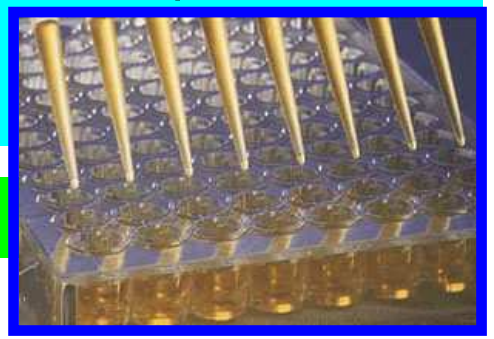
Campioni in doppio dello stesso paziente raccolti nello stesso momento:

- LCR: almeno 0,5 mL, preferibilmente 1-2 mL per eventuali ripetizioni
- Siero: almeno 0,5 mL

Le coppie di campioni (LCR e siero) possono essere conservati per 14 gg. a 2-8℃ prima dell'indagine e sino a 6 mesi a -20℃ o ad una T. inferiore

Antigene: flagello nativo purificato DK1 del ceppo *B. afzelii,* altamente immunogenico, induce una immunorisposta precoce, intensa e persistente.

- maggiore sensibilità e specificità diagnostica
- idoneo in tutte le aree geografiche



Tecnica: ELISA competitivo

IDEIA™ Lyme Neuroborreliosis

SOMMARIO DELLA PROCEDURA OPERATIVA DEL SAGGIO IDEIA™ LYME NEUROBORRELIOSIS

Attendere che tutti i reagenti raggiungano la temperatura ambiente (15–30°C) prima dell'uso

Aggiungere 100µL di controllo o di CSF/siero del test

↓

Coprire e incubare a 20–25°C con agitazione per 60 minuti

↓

Lavare (4 volte)

↓

Aggiungere 100µL di coniugato del flagello **FLAGELLUM CONJUGATE**

↓

Coprire e incubare a 20–25°C con agitazione per 60 minuti

↓

Lavare (4 volte)

↓

Aggiungere 100µL di substrato **SUBSTRATE TMB**

↓

Coprire e incubare a 20-25°C senza agitazione per 10 minuti

↓

Aggiungere 100µL di soluzione bloccante **STOP SOLUTION**

↓

Leggere l'assorbanza fotometricamente a 450nm (valore di riferimento 620-650nm)

IDEIA™ Lyme Neuroborreliosis

La produzione intratecale di Ab è teoricamente presente se ODCSF/ODSiero >1.
La differenza di OD netta (ODCSF - ODSiero) dà informazioni più precise.
Tuttavia, a causa delle variazioni intra-saggio, la differenza minima di densità ottica indicativa della sintesi intratecale di Ab aumenta con i valori di densità ottica.
Problemi eliminati moltiplicando il rapporto OD per la differenza OD espressa dall'indice di anticorpo specifico (I).
Il limite inferiore dell'indice pari a 0,3 per un risultato positivo assicura che ODCSF sia significativamente maggiore di ODSiero

Risultati positivi
Un indice ≥0,3 per le IgG e/o le IgM con concomitante pleocitosi mononucleare nel CSF è fortemente indicativo della neuroborreliosi di Lyme.
Un indice ≥0,3 per le IgM è generalmente compatibile con una durata della malattia < 6 mesi, tuttavia l'individuazione delle IgM non è determinante. I pazienti con NBL attiva di durata > 6 mesi presentano generalmente la sintesi intratecale solamente di IgG specifiche. In questi pazienti viene rilevato un indice ≥0,3 per le IgG.

Risultati negativi
Un risultato negativo, con indice <0,3 per IgG e per IgM, con concomitante pleocitosi mononucleare nel CSF, non esclude la diagnosi clinica di NBL.
Nella maggior parte dei casi non trattati con segni clinici di NBL, gli Ab specifici risultano individuabili nel CSF nella 2ª settimana dal manifestarsi dei sintomi

12 LIMITAZIONI DI PERFORMANCE

12.1 Una contaminazione sanguigna eccessiva nel campione di CSF può portare a indici di anticorpi specifici falsamente bassi.

12.2 A causa della relazione antigenica tra *B. burgdorferi* e *Treponema pallidum*, si può manifestare, sebbene raramente, una cross-reattività sierologica in pazienti con una storia recente o passata di neurosifilide. Una discriminazione sierologica è possibile utilizzando il saggio di emoagglutinazione del *T. pallidum* (TPHA) oppure i seguenti testi alla cardiolipina non treponemali: VDRL, test rapido della reagina del plasma (RPR) e reazione di Wassermann. Questi test risultano negativi nei pazienti affetti solamente da infezione da *B. burgdorferi.*

12.3 Una precedente terapia antibiotica può sopprimere la risposta anticorpale, rendendo i risultati sierologici meno predittibili.

12.4 La raccolta di campioni in uno stadio precoce della malattia può portare a risultati negativi (vedi Sezione 11).

12.5 Qualsiasi alterazione dei reagenti o la conservazione non conforme a quanto indicato nella Sezione 5.2 può influire negativamente sul risultato del test.

12.6 I risultati del test devono essere interpretati congiuntamente ad altre informazioni provenienti da studi epidemiologici, alla valutazione clinica del paziente e ad altre procedure diagnostiche.

Diagnosis of Lyme Borreliosis

Aguero-Rosenfeld ME., et al. Clin Microbiol Rev 2005 Jul,18(3):484-509

TABLE 3. Sensitivities and specificities of PCR assays for detection of *B. burgdorferi* DNA in different clinical specimens from patients with LB[a]

Clinical specimen and region	No. of studies included	Median % sensitivity[b] (range)	Reported % specificity range	References
Skin biopsy				
EM	16	69 (36–88)	98–100	
United States	4	64 (59–67)	98–100	177, 301, 303, 327
Europe	12	73 (36–88)	100	31, 165, 209–211, 234, 256, 272, 279, 344, 358, 359
ACA, Europe	8	76 (54–100)	100	31, 199, 209, 210, 256, 279, 344, 359
Blood, plasma, serum	6	14 (0–100)		
United States	3	18 (0–59)	100	108, 172, 345
Europe	3	10 (4–100)	NA[c]	78, 233, 234
CSF	16	38 (12–100)	93–100	
United States	6	73 (25–93)	93–100	134, 150, 172, 180, 223, 239
Europe	10	23 (12–100)	98–100	11, 57, 74, 88, 130, 162, 233, 236, 270, 377
Synovial fluid	8	78 (42–100)	100	
United States	4	83 (76–100)	100	30, 172, 224, 252
Europe	4	66 (42–85)	100	87, 133, 270, 293

[a] Only studies published in MEDLINE-indexed periodicals during the years 1991 to 2003 and those examined by PCR assay for ≥5 cases are included.

[b] Median sensitivity of PCR assays based on included studies. For studies tested with multiple PCR primer sets, the highest sensitivity reported was selected for analysis.

[c] NA, not available.

EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis

Mygland A, et al. Eur J Neurol. 2010 Jan;17(1):8-16, e1-4

PCR

There are numerous PCR protocols for detection of *Bb* DNA in clinical specimens [1,33,34]. Because of the lack of a gold standard method and lack of large comparative studies, at present it is impossible to recommend a specific PCR protocol. Diagnostic sensitivity of PCR in CSF for early LNB is 10–30% (median). In blood it is even lower [35], and PCR studies in urine are contradictory [1,33,36–38]. In late LNB, sensitivity of PCR is extremely low.

Recommendation. PCR on CSF samples has a low sensitivity, but may be useful in very early LNB with negative antibody index (AI), or in patients with immunodeficiency (GPP). Because of low sensitivity and unknown specificity, PCR cannot be recommended as a diagnostic method in patients with chronic symptoms or for follow-up of therapy.

Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue

Babady NE, et al. Diagn Microbiol Infect Dis 2008 62: 464–466

Table 2
Lyme PCR positivity rates by specimen source

Results	Specimen source				
	Blood	CSF	SF	Tissue[a]	Others[b]
Positive	6	14	127	6	1
Negative	5697	15 925	1849	86	66
Total	5703	15 939	1976	92	67
Percent positive rate (%)	0.1	0.09	6.4	6.5	1.5

[a] Positive tissue sources include knee, arm, leg, stomach, back, and thigh.

[b] Other sources include pleural fluid, pericardial fluid, ventricular fluid, vitreous fluid, urine, and leg abscess.

Presence of anti-Borrelia burgdorferi antibodies and Borrelia burgdorferi sensu lato DNA in samples of subjects in an area of the Northern Italy in the period 2002–2008

Calderaro A, et al. Diagn Microbiol Infect Dis 2011 70; 455–460

No. of patients	First-step assay			Confirmatory assay	
	EIA	IFA		WB	
	No. of		Result	No. of samples	Result

PCR assay did not detect *B. burgdorferi* DNA in any of the 194 analyzed specimens belonging to 138 patients except for a tick (adult male *I. ricinus*) collected from the skin of a patient (who remained healthy during 18 months follow-up) whose available samples (serum, blood, urine) resulted negative both by serologic (serum) and PCR assays (all samples).

				1	IgG− IgM−
1	1	0	IgG+/− IgM+	1	IgG+ IgM+
2	2	0	IgG+/− IgM−	2	IgG+ IgM−
19	19	0	IgG− IgM+/−	2	IgG− IgM+/−
				17	IgG− IgM−
2017	523	1494	IgG− IgM−	0	Not done
Total				319	
2336	565	1771			

GeneProof®

Borrelia burgdorferi (BB)

PCR Kit

CE

in vitro Diagnostics

Individual Report

Dataset code : 1 **Date Submitted : 24 NOV 11**

QCMD
Quality Control for Molecular Diagnostics

Expected results of the programme in order of sample content and concentration.

Sample	Matrix *	Sample Content	Sample conc. †
BbDNA11-01	Buffer	B. burgdorferi DNA	1.0E+06 Copies/ml
BbDNA11-03	Buffer	B. burgdorferi DNA	1.0E+05 Copies/ml
BbDNA11-02	Buffer	B. burgdorferi DNA	1.0E+05 Copies/ml
BbDNA11-04	Buffer	B. burgdorferi DNA	1.0E+04 Copies/ml
BbDNA11-05	Buffer	B. burgdorferi DNA Negative	
BbDNA11-06	Culture medium	B. burgdorferi	1.0E+05 cells/ml
BbDNA11-08	Culture medium	B. burgdorferi	1.0E+04 cells/ml
BbDNA11-10	Culture medium	B. burgdorferi	1.0E+04 cells/ml
BbDNA11-07	Culture medium	B. burgdorferi	1.0E+03 cells/ml
BbDNA11-09	Culture medium	B. burgdorferi negative	

* Buffer: TE Buffer. Culture medium: Borrelia MKP medium.

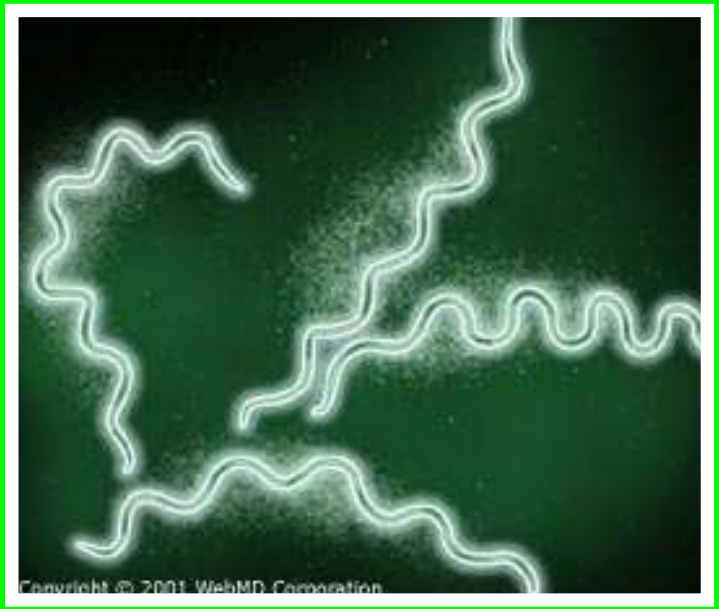
† Copies/ml or cells/ml. The values are not technology specific and should not be used by participants for method comparison or as a target for individual laboratory assessment.

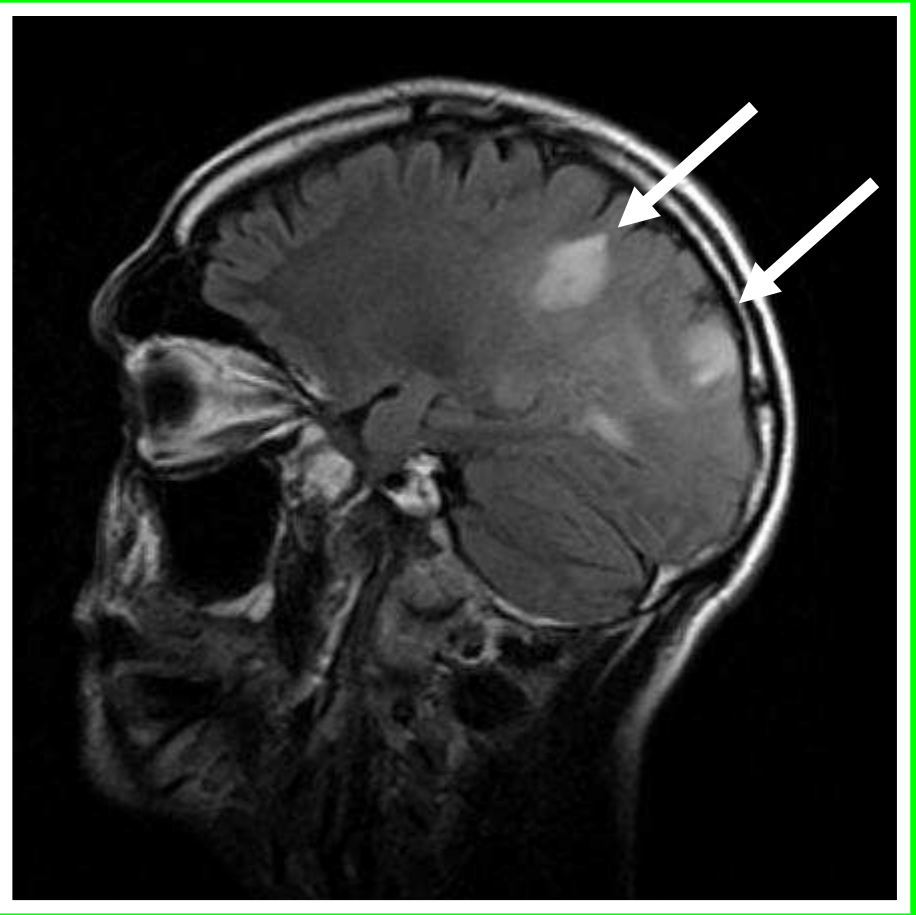
Your laboratory's qualitative results and performance scores

Sample	Sample Content	Qualitative			
		Sample Status	Sample Type	Your qualitative result	Your qualitative score
BbDNA11-01	B. burgdorferi DNA	Frequently detected	Core	positive	0
BbDNA11-03	B. burgdorferi DNA	Frequently detected	Core	positive	0
BbDNA11-02	B. burgdorferi DNA	Frequently detected	Core	positive	0
BbDNA11-04	B. burgdorferi DNA	Detected		positive	0
BbDNA11-05	B. burgdorferi DNA Negative	Negative	Core	negative	0
BbDNA11-06	B. burgdorferi	Frequently detected	Core	positive	0
BbDNA11-08	B. burgdorferi	Frequently detected	Core	positive	0
BbDNA11-10	B. burgdorferi	Frequently detected	Core	positive	0
BbDNA11-07	B. burgdorferi	Frequently detected		positive	0
BbDNA11-09	B. burgdorferi negative	Negative	Core	negative	0
Sum Qualitative Panel Score					0

The core proficiency samples in this EQA programme were:
BbDNA11-01, BbDNA11-02, BbDNA11-03, BbDNA11-05, BbDNA11-06, BbDNA11-08, BbDNA11-09, BbDNA11-10
You reported 8/8 (100.0 %) of the core samples correctly.
Of the total datasets reported by all participants in this EQA programme, 94.1% reported correct results for all core proficiency samples.

Treponema pallidum

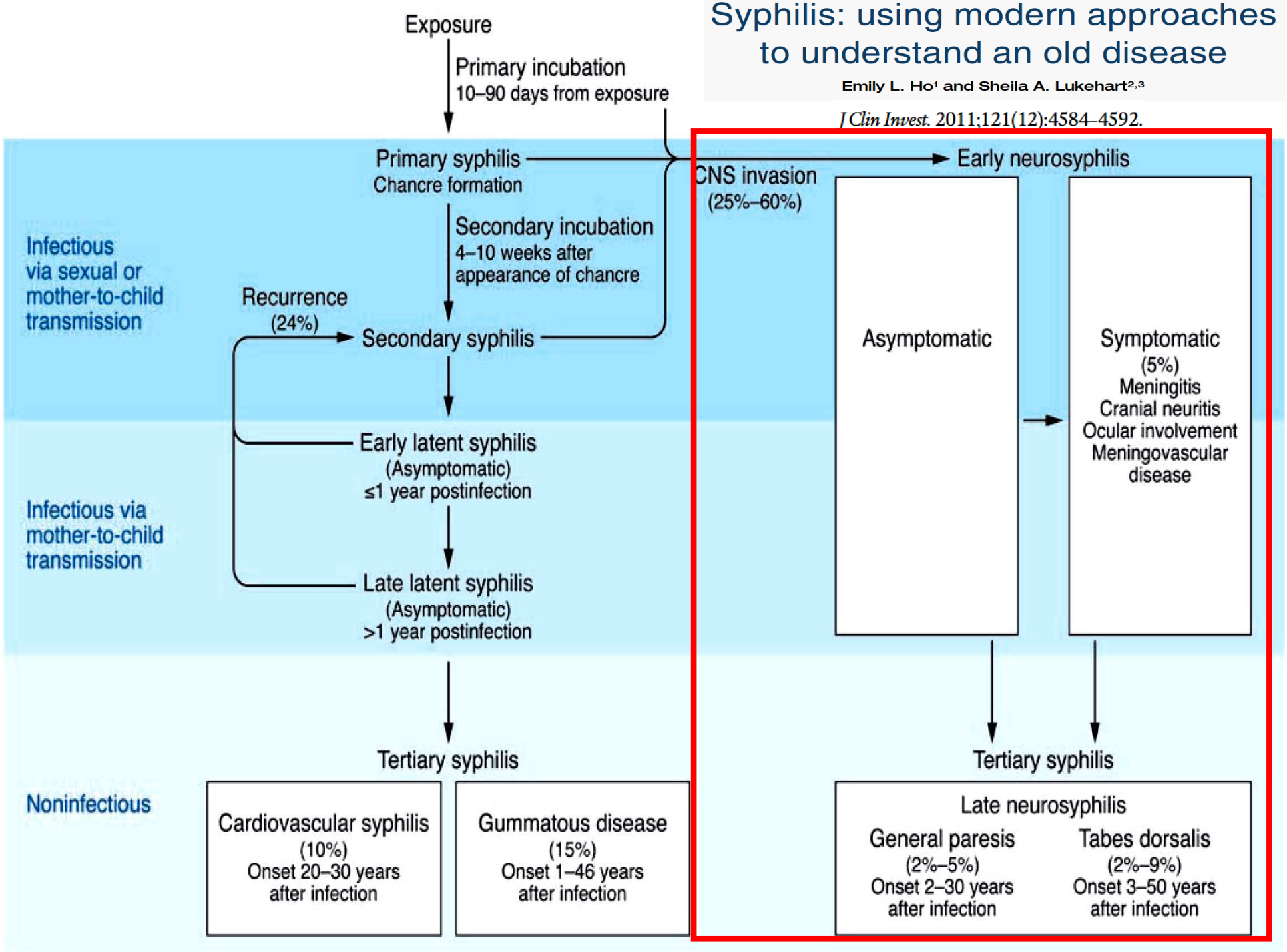




Syphilis: using modern approaches to understand an old disease

Emily L. Ho[1] and Sheila A. Lukehart[2,3]

J Clin Invest. 2011;121(12):4584–4592.



Which algorithm should be used to screen for syphilis?

Binnicker MJ. Curr Opin Infect Dis 2012 Feb;25(1):79-85 Review

Purpose of review

A growing number of laboratories have implemented a reverse screening algorithm for syphilis testing, which has created confusion among many healthcare providers. This review focuses on recent data addressing the clinical and economical impact of reverse screening and discusses the advantages and limitations of the traditional and contemporary algorithms.

Recent findings

Screening for syphilis using a treponemal assay detects a higher number of patients with reactive results compared to traditional screening by rapid plasma reagin (RPR). Furthermore, a significant percentage of patients who are reactive by a treponemal screening assay are nonreactive by RPR. These discordant results may occur in patients with past, treated or untreated syphilis; early syphilis; or no syphilis. Recent reports suggest that the reverse screening algorithm may result in increased patient follow-ups, overtreatment, and potentially higher cost. However, other data suggest that reverse screening facilitates the detection of latent and early syphilis, while offering an objective and automated screening approach.

Summary

The Centers for Disease Control and Prevention currently recommends syphilis screening with a nontreponemal test. However, as laboratories continue to implement the reverse screening algorithm, it is important that samples with discordant screen-reactive, RPR-nonreactive results be tested by a second treponemal assay to assist in the interpretation of results.

Which algorithm should be used to screen for syphilis?

Binnicker MJ. Curr Opin Infect Dis 2012 Feb;25(1):79-85 Review

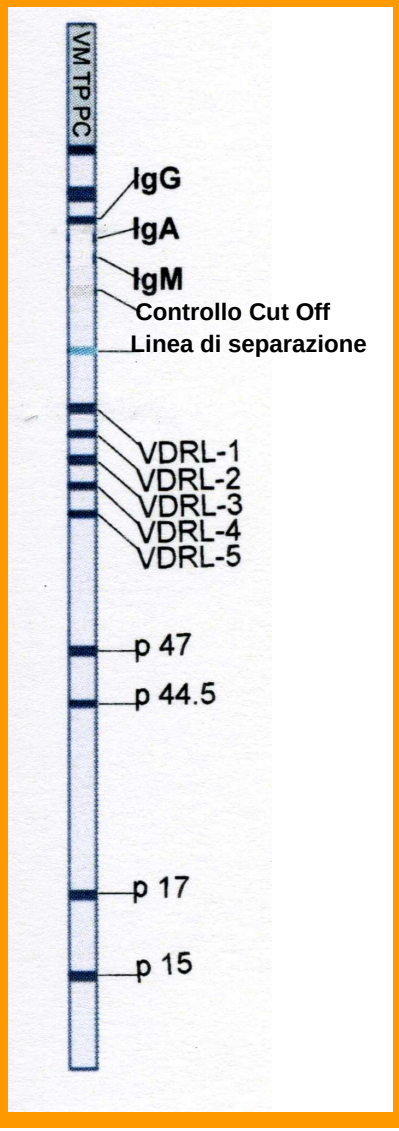
Table 1. Interpretation and follow-up of reverse screening results

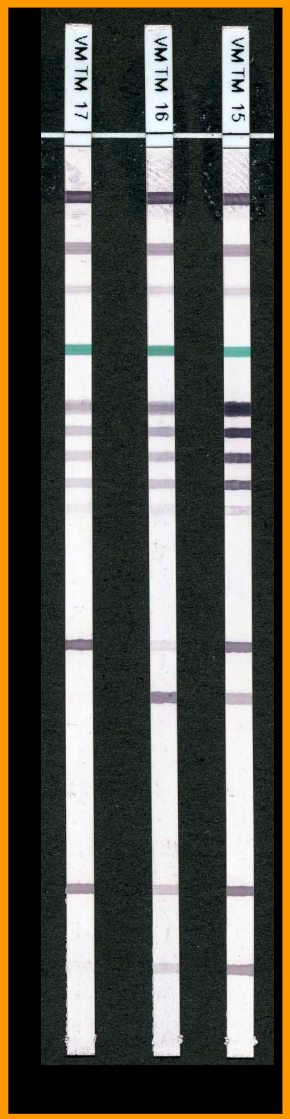
Patient history	Test and result: EIA/CIA/MFI	Test and result: RPR	Test and result: TP-PA	Interpretation	Follow-up
Unknown history of syphilis	Nonreactive	N/A	N/A	No serologic evidence of syphilis	None, unless clinically indicated (e.g. early syphilis)
Unknown history of syphilis	Reactive	Reactive	N/A	Untreated or recently treated syphilis	See CDC treatment guidelines[a]
Unknown history of syphilis	Reactive	Nonreactive	Nonreactive	Probable false-positive screening test	No follow-up testing, unless clinically indicated
Unknown history of syphilis	Reactive	Nonreactive	Reactive	Possible syphilis (e.g. early or latent) or previously treated syphilis	Historical and clinical evaluation required
Known history of syphilis	Reactive	Nonreactive	Reactive or N/A	Past, successfully treated syphilis	None

CIA, chemiluminescence immunoassay; EIA, enzyme immunoassay; MFI, multiplex flow immunoassay; N/A, not applicable; RPR, rapid plasma reagin; TP-PA, *Treponema pallidum* particle agglutination.

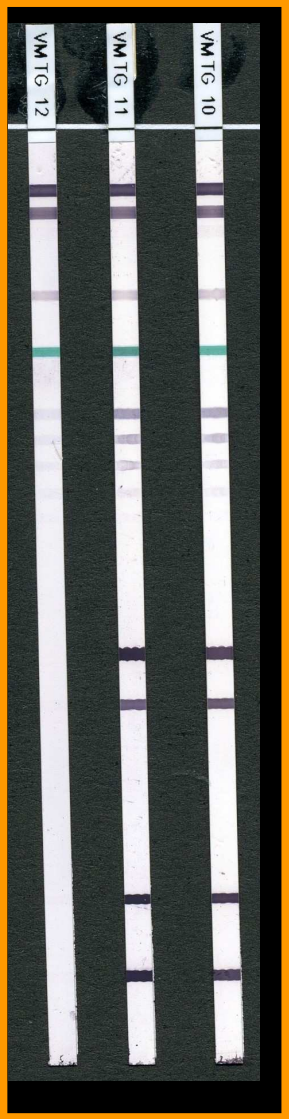
WESTERN BLOT

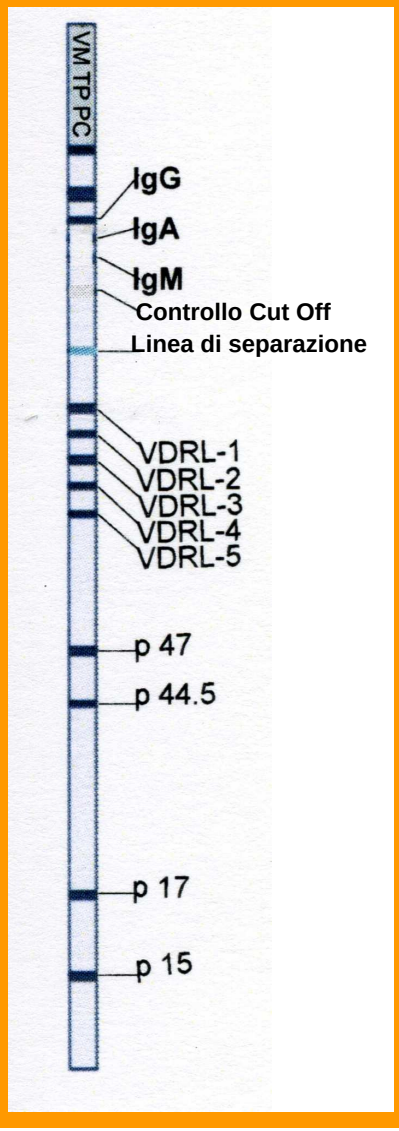
IgM





IgG





Evaluation of rapid diagnostic tests: syphilis

NATURE REVIEWS | MICROBIOLOGY
DECEMBER 2006

WHO/TDR Sexually Transmitted Diseases Diagnostics Initiative

	Non-treponemal tests		Treponemal tests		
Test characteristic	**RPR**	**VDRL**	**EIA**	**TPPA/TPHA**	**FTA-ABS**
Specimen	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma
Sensitivity	86–100%	78–100%	82–100%	85–100%	70–100%
Specificity	93–98%	98–100%	97–100%	98–100%	94–100%
Ease of use	Easy	Easy	Moderate	Complex	Complex
Level of use	Exam room, on-site lab	Exam room, on-site lab	Intermediate lab, reference lab	Reference lab	Reference lab
Equipment required	Rotator, refrigerator	Light microscope, refrigerator	Incubator, microwell plate washer and reader	Incubator	Fluorescence microscope
Training	Minimal	Minimal	Moderate	Extensive	Extensive
Average cost	US$ 0.5	US$ 0.5	US$ 3	US$ 3	US$ 3
Comments	Reagents require refrigeration	Reagents require refrigeration	Allows high-throughput screening; does not distinguish between prior treated and active infection	Used as confirmatory test; does not distinguish between prior treated and active infection	Used as confirmatory test; does not distinguish between prior treated and active infection

The Rapid Plasma Reagin Test Cannot Replace the Venereal Disease Research Laboratory Test for Neurosyphilis Diagnosis

Marra CM, et al. Sex Transm Dis. 2012 Jun;39(6):453-7.

TABLE 3. Sensitivity and Specificity of the 3 CSF Nontreponemal Serological Tests for Diagnosis of Neurosyphilis

	Diagnostic Criterion			
	Laboratory-Defined Neurosyphilis (n = 99)		Symptomatic Neurosyphilis (n = 149)	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)*
CSF-VDRL	71.8 (57.7–85.9)	98.3 (95.0–100.0)	66.7 (50.6–82.8)	80.2 (72.9–87.5)
CSF-RPR	56.4 (40.8–72.0)	100.0 (100.0–100.0)	51.5 (34.4–68.6)	89.7 (84.2–95.2)
CSF-RPR-V	59.0 (43.6–74.4)	98.3 (95.0–100.0)	57.6 (40.7–74.5)	84.5 (77.9–91.1)

Patients with laboratory-defined neurosyphilis had reactive CSF-FTA-ABS and CSF WBCs >20/uL. Patients with symptomatic neurosyphilis had vision loss or hearing loss.

*For diagnosis of symptomatic neurosyphilis, the CSF-RPR was significantly more specific than the CSF-VDRL ($P = 0.04$).

ABS indicates absorption; CSF, cerebrospinal fluid; FTA, fluorescent treponemal antibody; RPR, rapid plasma reagin; RT-PCR, reverse transcriptase-polymerase chain reaction; VDRL, Venereal Disease Research Laboratory; WBC, white blood cells.

The Performance of Cerebrospinal Fluid Treponemal-Specific Antibody Tests in Neurosyphilis: A Systematic Review

Harding AS, et al. Sex Transm Dis. 2012 Apr;39(4):291-7. Review.

Background: No single laboratory test is both sensitive and specific to diagnose neurosyphilis. Several major clinical guidelines suggest that negative cerebrospinal fluid (CSF) treponemal-specific antibody tests rule out the diagnosis of neurosyphilis. Our aim was to systematically review the literature and describe the performance of treponemal-specific CSF antibody tests when diagnosing neurosyphilis.

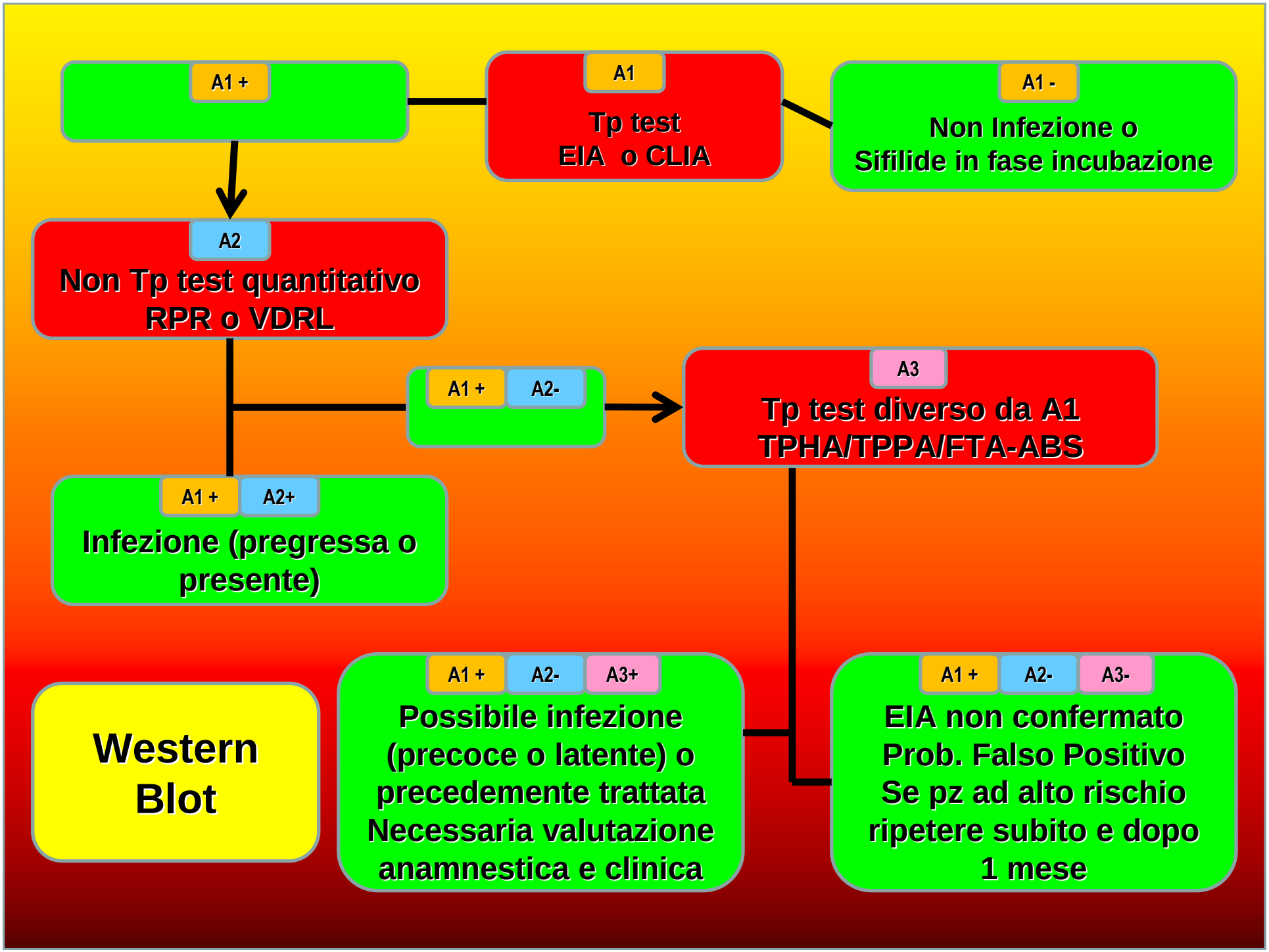
The most widely used test, the nontreponemal Venereal Disease Research Laboratory (VDRL) CSF test, is generally considered to have poor sensitivity but high specificity in diagnosing neurosyphilis.[2]

The Performance of Cerebrospinal Fluid Treponemal-Specific Antibody Tests in Neurosyphilis: A Systematic Review

Harding AS, et al. Sex Transm Dis. 2012 Apr;39(4):291-7. Review.

Our study highlights several important issues. Prospective studies with a clinically meaningful definition of neurosyphilis are needed to better assess the utility of these CSF diagnostics. A positive CSF VDRL may be the most specific test in diagnosing neurosyphilis, but using it as the sole criterion to define neurosyphilis may affect a study's clinical applicability since 50% of persons treated for neurosyphilis in clinical practice have a negative CSF VDRL. More research is needed in the application of enzyme immunoassay (EIA) studies for use in the CSF,[26] in defining the role of treponemal-specific antibody titer levels or indices in the diagnosis of neurosyphilis,[21] and in the discovery of novel biomarkers for neurosyphilis.[27]

Conclusions: A negative CSF-treponemal-specific antibody test may not exclude the diagnosis of neurosyphilis when the clinical suspicion for neurosyphilis is high.


A1 +
A1
Tp test
EIA o CLIA
A1 -
Non Infezione o
Sifilide in fase incubazione
A2
Non Tp test quantitativo
RPR o VDRL
A1 +
A2-
A3
Tp test diverso da A1
TPHA/TPPA/FTA-ABS
A1 +
A2+
Infezione (pregressa o
presente)
Western
Blot
A1 +
A2-
A3+
Possibile infezione
(precoce o latente) o
precedemente trattata
Necessaria valutazione
anamnestica e clinica
A1 +
A2-
A3-
EIA non confermato
Prob. Falso Positivo
Se pz ad alto rischio
ripetere subito e dopo
1 mese

PROTOCOLLO DI SORVEGLIANZA DELLA SIFILIDE CONGENITA

Azienda Ospedaliero-Universitaria di Bologna

Neonati nati da madri con sierologia positiva per lue

Nati da madri con trattamento effettuato prima della gravidanza o comunque > 30 gg prima del parto

→ Esecuzione RPR quantitativo, TPHA e WB IgG/IgM

Nati da madri mai trattate o con trattamento inadeguato (< 30 gg prima del parto o con farmaci diversi dalla penicillina)

→ Esecuzione RPR quantitativo, TPHA e WB IgG/IgM su siero e VDRL, TPHA e WB IgG/IgM su liquor

TUTTI i neonati vengono inseriti nel follow up.
Secondo protocollo sono previsti prelievi seriali a 3, 6, 9, 12 mesi e/o fino a eventuale negativizzazione dei test treponemici.

Development of a Real-Time PCR Assay To Detect *Treponema pallidum* in Clinical Specimens and Assessment of the Assay's Performance by Comparison with Serological Testing

Leslie DE, et al. *J Clin Microbiol* 2007 Jan;45(1):93-6

TaqMan real-time PCR assay targeting the *polA* gene of *Treponema pallidum* – *HOME MADE*

TABLE 3. Comparison of PCR results against serology for 301 patients with adequate serological testing

PCR result	Serology result (no. of patients)		
	Positive	Negative	Total
Positive	41	4	45
Negative	10	246	256
Total	51	250	301

Directly compared with serology, TpPCR showed 95% agreement, with a sensitivity of 80.39% and a specificity of 98.40%.

We found that TpPCR is a useful addition to serology for the diagnosis of infectious syphilis.

Development of a Real-Time PCR Assay To Detect *Treponema pallidum* in Clinical Specimens and Assessment of the Assay's Performance by Comparison with Serological Testing

Leslie DE, et al. J Clin Microbiol 2007 Jan;45(1):93-6

TABLE 2. TpPCR results by site of sampling

Sample site	No. (%) of:			
	All specimens		Specimens with adequate serology available for comparison	
	Total	TpPCR positive	Total	TpPCR positive
Penile	195	30 (15.4)	102	26
Anorectal, perianal, etc.	152	11 (7.2)	80	8
Oropharyngeal, tongue, lip, etc.	69	6 (8.7)	39	6
Other superficial body site	30	2 (6.7)	15	1
Genital swab, not further specified	20	2 (10.0)	13	2
Site not stated	38	2 (5.3)	11	2
Other deep site	9	2[a] (22.2)	0	0
Groin, scrotum, pubis, perineum	21	0 (0.0)	7	0
Urethral	34	0 (0.0)	20	0
Vulvovaginal, cervix	62	0 (0.0)	14	0
Cerebrospinal fluid	30	0 (0.0)	0	0
Total	660	55 (8.3)	301	45

Clinical Value of *Treponema pallidum Real-Time PCR for* Diagnosis of Syphilis

Heymans R, et al. J Clin Microbiol 2010; 48 (2): 497-502

(iii) *T. pallidum* real-time PCR. For testing by the *T. pallidum* real-time PCR, two dry swab specimens were obtained from the (ano)genital ulcer or skin scraping. Within 24 h, these swabs were transported to the Public Health Lab-

TABLE 4. Clinical diagnosis of primary syphilis made by using syphilis serology and patient history as a model for a general practitioner setting versus the result of the *T. pallidum* real-time PCR[a]

T. pallidum real-time PCR result	No. of samples with the following result for clinical diagnosis in GP setting[b]:	
	+	−
+	76	17
−	26	597

[a] The data are for a total of 716 cases. The sensitivity of the *T. pallidum* real-time PCR was 75%, and its specificity was 97%. The kappa value was 0.745, which indicates good agreement between the results of the two tests.

[b] Primary syphilis was diagnosed either in patients with a positive TPPA result (irrespective of the RPR test result) without a history of syphilis or in patients with an RPR titer of ≥1:8 and a history of syphilis (Fig. 2).

Clinical Value of *Treponema pallidum Real-Time PCR for* Diagnosis of Syphilis

Heymans R, et al. J Clin Microbiol 2010; 48 (2): 497-502

TABLE 6. Diagnosis of secondary syphilis made on the basis of suspected skin or mucosal findings plus an RPR titer of ≥1:8 versus the result of the *T. pallidum* real-time PCR[a]

T. pallidum real-time PCR result	No. of samples with the following result for secondary syphilis:	
	+	−
+	33	1
−	44	55

[a] The data are for a total of 133 cases. The sensitivity of the *T. pallidum* real-time PCR was 43.0%, and the specificity was 98.0%. The kappa value was 0.372, which indicates slight agreement between the results of the two tests.

Evaluation of a PCR Test for Detection of Treponema pallidum in Swabs and Blood

Grange PA, et al. J Clin Microbiol 2012 Mar;50(3):546-52

TABLE 4 Statistical analysis of nPCR detection as a function of the HIV status of the patient

	No. of HIV-negative patients with:											No. of HIV-positive patients with:										
	Primary syphilis		Secondary syphilis		Latent syphilis		No syphilis					Primary syphilis		Secondary syphilis		Latent syphilis		No syphilis			
Sample type	Total	nPCR positive	Total	nPCR positive	Total	nPCR positive	Total	nPCR positive	Se	Sp	Total	nPCR positive	Total	nPCR positive	Total	nPCR positive	Total	nPCR positive	Se	Sp	*P* value
Swab	47	39	30	25			25	1	83	96	33	26	39	28			15	2	76	93	1.00
PBMC	38	12	35	12	10	0	26	1	28	96	28	10	36	11	14	4	15	0	31	100	0.624
Plasma	33	4	28	5	9	1	20	1	16	95	28	3	29	10	12	1	8	0	20	100	0.384
Serum	35	10	31	3	13	1	24	2	17	91	24	2	30	7	13	0	9	0	13	100	0.521
Whole blood	37	3	31	11	12	3	22	1	20	95	19	3	28	10	11	1	8	0	24	100	0.543

Diagnóstico de la infección por *Treponema pallidum* en pacientes con sífi lis temprana y neurosífi lis mediante reacción de la polimerasa en cadena

Garcia P, et al. Rev Chil Infect 2011; 28 (4): 310-315

Tabla 1A. Validación de la RPC para detección de *T. pallidum* en lesiones muco-cutáneas en base a diagnóstico clínico de sífilis temprana por estándar de oro ampliado

		Diagnóstico clínico de ST por		
		Positivo	Negativo	Total
Reacción de la polimerasa cadena (RPC)	Negativo	1	12	13
	Total	21	12	33
Sensibilidad: 20/21=95%;	Especificidad: 12/12=100%	VPP: 20/20=100%	VPN: 12/13=92%	

The reaction was done using primers targeting the *tpN47*gene. HOME MADE

Tabla 1B. Validación de la RPC para detección de *T. pallidum* en LCR en base a diagnóstico clínico de neuro sífilis por estándar de oro ampliado

		Diagnóstico clínico de NS por estándar de oro ampliado		
		Positivo	Negativo	Total
Reacción de la polimerasa en cadena (RPC)	Positivo	4	0	4
	Negativo	4	23	27
	Total	8	23	31
Sensibilidad: 4/8 = 50%	Especificidad: 23/23 = 100%	VPP: 4/4=100%	VPN: 23/27=85%	

Seegene

Infectious Pathogen Detection

STI MASTER ACE DETECTION
Multiplex PCR

Panel 5
for diagnosis of soft-chancroid, hard-chancroid, ulcer and GBS

CMV

Treponema pallidum

Haemophilus ducreyi

Group B Streptococcus (GBS)

Lymphogranuloma venereum (LGV)

Specimens:
Urine
Genital swab
Liquid-based cytology specimen

Molecular Subtyping of *Treponema pallidum* during a Local Syphilis Epidemic in Men Who Have Sex with Men in Melbourne, Australia

Azzato F, et al. J Clin Microbiol 2012 Jun;50(6):1895-9.

Clinical specimens. Specimens tested included oral, genital, and anorectal ulcer or lesion swab, cerebrospinal fluid (CSF), and tissue biopsy specimens.

DNA extraction. Total DNA was extracted from fresh specimens on an Xtractor system (Qiagen Hilden, Germany) using a DX reagent pack, as per the manufacturer's instructions. DNA was extracted from paraffin-embedded tissue sections as described by Fyfe et al. (6).

Real-time PCR for detection of *T. pallidum*. A TaqMan real-time PCR assay targeting the *polA* gene of *T. pallidum* as described by Leslie et al. was used to screen for *T. pallidum* (9).

Molecular subtyping of *T. pallidum* from clinical samples. Samples that tested positive for the *polA* gene of *T. pallidum* were retrospectively subtyped using the method described by Pillay et al. (14)

Molecular Subtyping of *Treponema pallidum* during a Local Syphilis Epidemic in Men Who Have Sex with Men in Melbourne, Australia

Azzato F, et al. J Clin Microbiol 2012 Jun;50(6):1895-9.

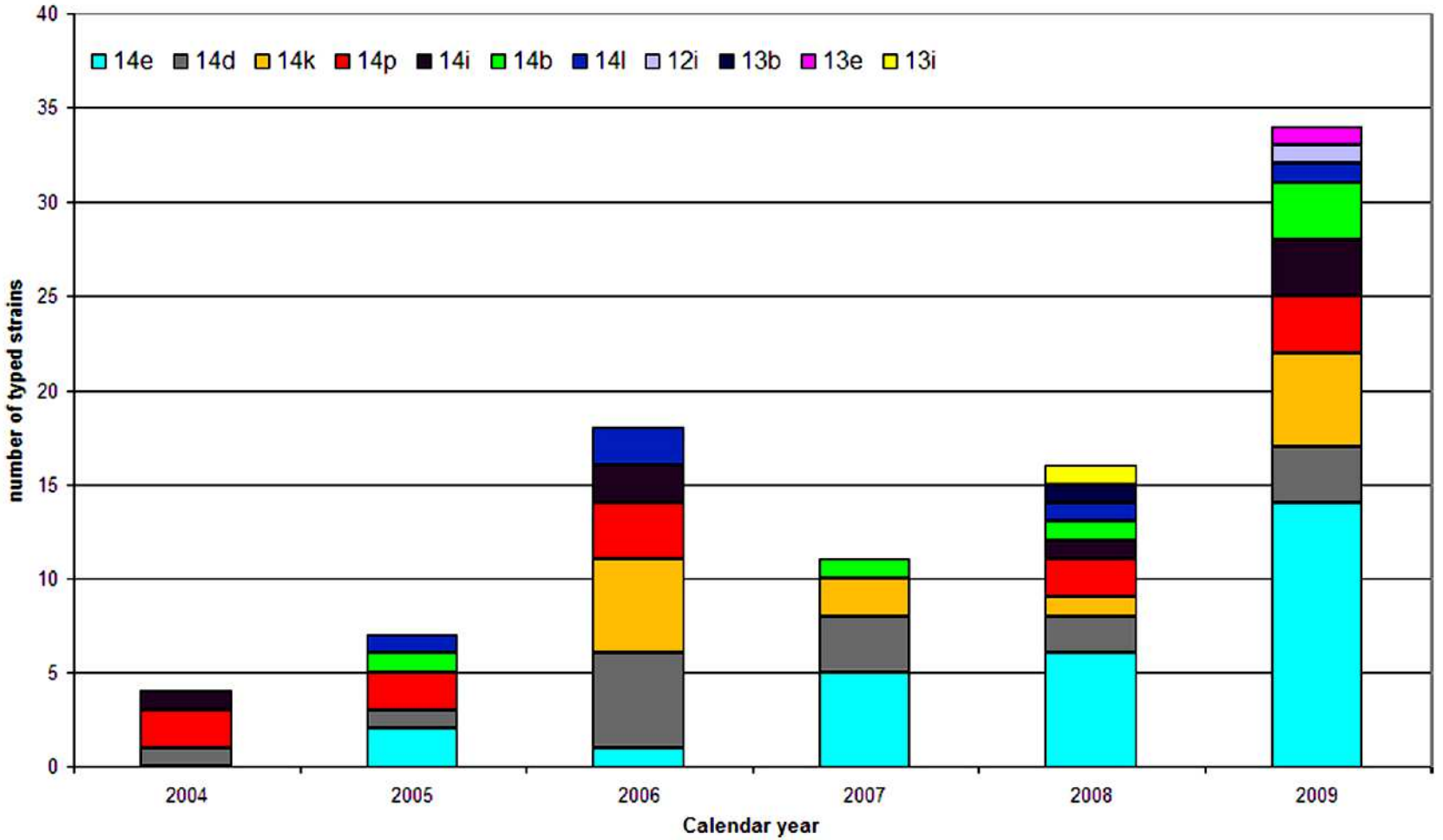


FIG 3 Distribution of *T. pallidum* subtypes, Victoria, Australia, 2004 to 2009. Data are for 90 specimens from 88 patients.

Molecular Subtyping of *Treponema pallidum* during a Local Syphilis Epidemic in Men Who Have Sex with Men in Melbourne, Australia

Azzato F, et al. J Clin Microbiol 2012 Jun;50(6):1895-9.

The 90 typeable samples were collected from the following sites anorectal (2 HIV, 15 non-HIV), penile or scrotal (11 HIV, 35 non-HIV), oropharyngeal (4 HIV, 7 non-HIV), and site of collection unknown (4 HIV and 12 non-HIV, with the latter including the single female patient in the study). Although the stage of in-


GRAZIE PER
L'ATTENZIONE

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