

19e symposium tuberculose de Munchenwiler  
25 Mars 2010

# Nucleic Acids Amplification Tests

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# Fast Diagnosis

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- Provides adequate treatment maximizing chances of success
- Breaks the chain of transmission contributing to TB control

# Microbiological Diagnosis: conventional and molecular

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- Relays on detection of AFB bacilli by microscopy and culture
  - Microscopy is rapid, but non specific for MTB and has low sensitivity
  - Culture is more sensitive, weeks to positivity and still negative in 10-20% of cases
- Relays on detection of MTB nucleic acids on the biological samples:
  - results in 2-6 hours
  - At present not suitable for therapy monitoring

# The problem with current testing methods

- (AFB) Smear test:
  - Insensitive, WHO and national guidelines recommend 2 to 3 smear tests to be performed by suspected patient
  - Requires skilled technician
  - Time consuming: 1-2 hours
- Culture
  - Highly sensitive
  - Slow: 3 to 6 weeks
- Nucleic Acid Amplification methods:
  - Highly specific
  - Not optimal on clinical samples:
    - Highly sensitive on smear positive samples (95-100%)
    - Until now insufficient sensitivity on smear negative samples (60-75%)
  - Very sensitive on culture isolates:
    - For identification
    - For Rif susceptibility testing

# Nucleic Acid Amplification Tests

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- **Direct Amplification Test for MTB:**
  - Allow *direct* detection and identification of *M. tuberculosis* complex in clinical samples.
  - Some tests allow simultaneous detection of MDR-TB
  - Short TaT
  - Current guidelines recommend the use in combination with conventional methods.
- **For species ID:**
  - highly simplified species ID
- **For molecular characterization of strains:**
  - PCR based techniques speeded up time to results

*Biosafety level required depends on samples and technology*

# NAATs

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- Home-made
- Commercially developed
- Based on PCR or different technologies
- Fully/partially automated

# Summary of Commercial DATs for TB

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## **Amplicor/Cobas Amplicor (Roche)**

PCR 16SRNA

## **E-MTD (Gen-Probe)**

TMA of rRNA

## **BD ProbeTec ET (Becton-Dickinson)**

SDA IS6110 and 16s rRNA

## **RealArt MTB (Artus)**

Real Time-PCR

## **LAMP (Eiken Chemical)**

Isothermal amplification and visual readout with UV fluorescence

## **Genotype Myco direct (HainLS)**

LiPA assay/ lateral flow

## **InnoLiPARif TB (Innogenetics)**

## **MTBDRplus (Hain Lifesciences)**

## **GeneXpert TB ( Cepheid)**

# real-time PCR: advantages

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- *Time to results* ( different from TaT)
- *Wide range of target quantification*
- *Riduction of risk of amplicons contamination*
- *Detection of low number of target copies*

# GenoQuick<sup>®</sup> MTB

- Fast molecular genetic identification of the **M. tuberculosis complex** (MTBC) directly from pulmonary and extrapulmonary specimens
- DNA based Assay
- target: MTBC specific Analytical sensitivity: 500 – 1000 bacteria/ml
- Taq polymerase: HotStar Taq (QIAGEN)
- Equipment: centrifuge, water bath and thermal cycler

# Procedure

## GenoQuick® MTB

decontaminated specimen



DNA extraction



PCR



detection by  
lateral flow technology

## GenoQuick® MTB dipstick (10 min)



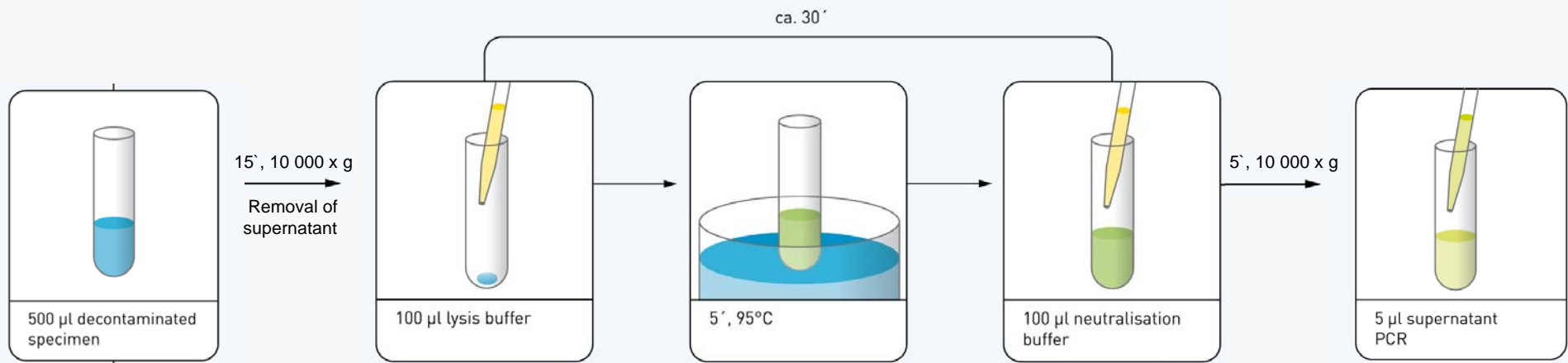
1: CC: Conjugate Control

2: AC: Amplification Control

3: MTBC

about 2 h 40 min

# Step 1: DNA extraction (GenoLyse<sup>®</sup>)

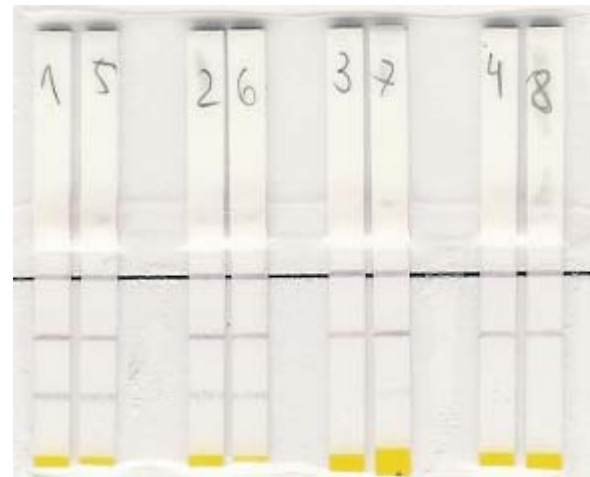


specimen → centrifugation → Cell lysis → 95°C incubation → neutralization → centrifugation → DNA

# Developed GenoQuick<sup>®</sup> MTB Dipsticks



- 1: CC: Conjugate Control
- 2: AC: Amplification Control
- 3. MTBC



- CC: Conjugate Control
- AC: Amplification Control
- MTBC

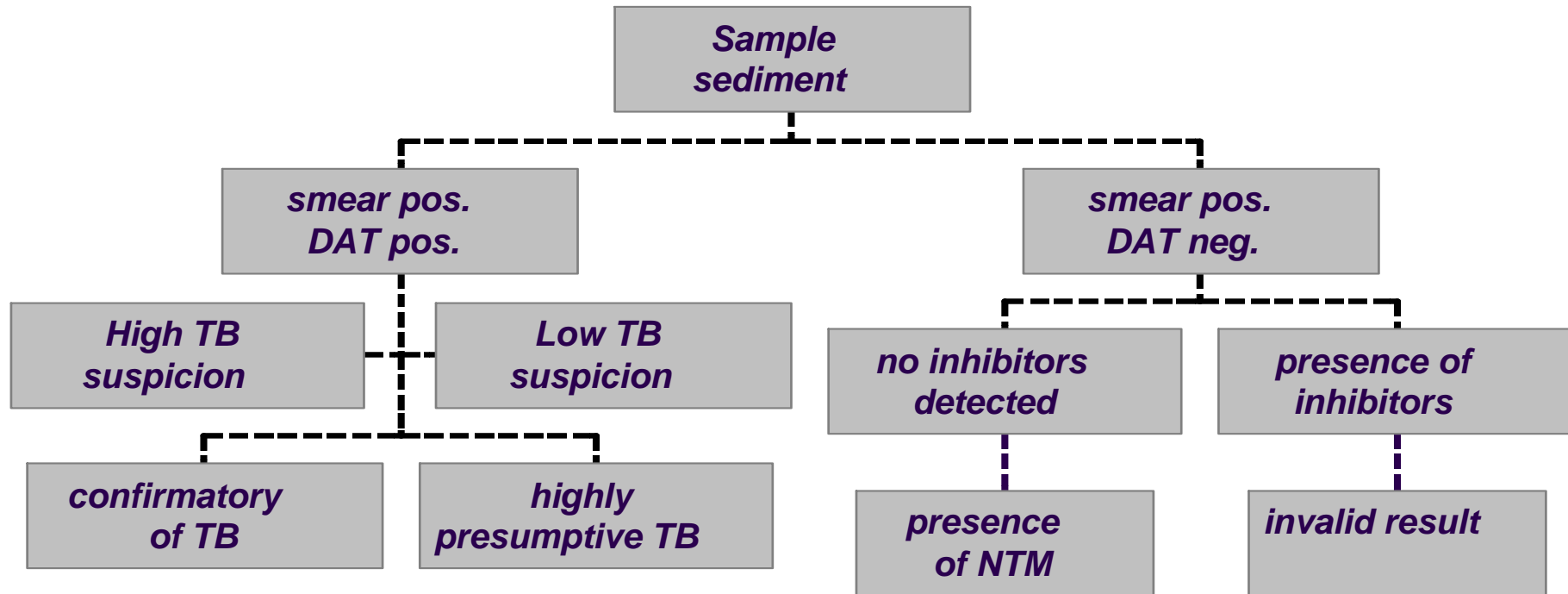
# FDA approved

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- Respiratory samples
  - With microscopy and culture
  - Untreated patients
  - AFB+
    - Amplicor E-MTD
  - AFB-
    - E-MTD

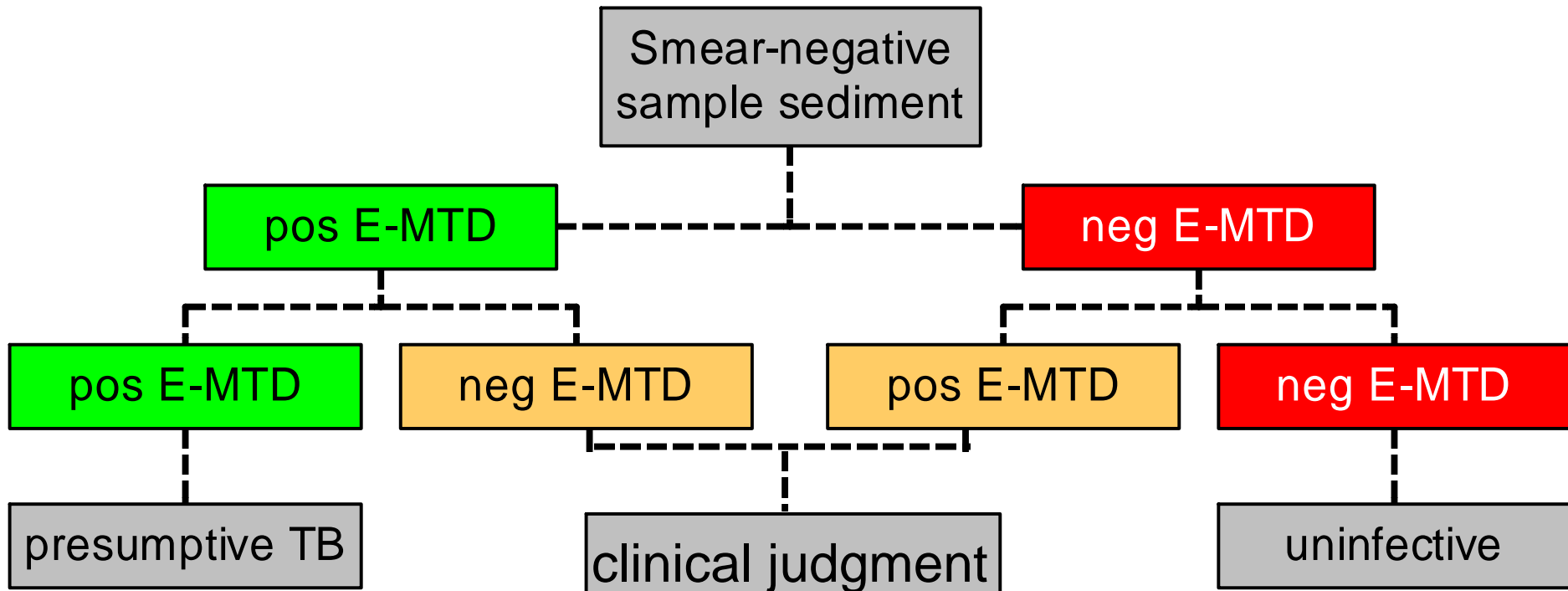
# Smear positive

MMWR 2000; 49: 593-594



# Smear negative

MMWR 2000; 49: 593-594



# New recommendations

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- “CDC recommends that Nucleic Acid Amplification testing 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, such as contact investigation

1. MMWR. Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis. Jan 19, 2009

# DAT and respiratory isolation protocols

Am.J.Respir.Crit.Care Med. 2008; 178: 300-305

## What This Study Adds to the Field

Single first-sputum NAA testing can rapidly and accurately detect all subjects considered “infectious” based on sputum smear analysis, and therefore has the potential to improve current respiratory isolation protocols.

	Tuberculosis* (n = 46)		No Tuberculosis (n = 447)
	Smear Positive (n = 35)	Smear Negative (n = 11)	
First-sputum NAA test result			
Positive	35	5	0
Negative	0	6	447
Sputum smear results			
Any of three smears positive	35	NA	17
First smear positive	32	NA	8

# When

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- High clinical suspect ( selection of patients)
  - *Clinical skills are uneven entities*
  - On the first sample
- results depend on
  - Sample quality and volume
  - Site of infection
- High suspect of MDR: simultaneous test for Rif res

# Selection of Patients

Clinical suspicion	No. of Patients	% Positive test		
		MTB culture	DAT*	MOTT culture
High	46	89	89	2
Medium	159	20	16	0.6
Low	56	0	<b>3.6</b>	0

Van den Wijngaert et al. JCM 2004; 42: 837-838

\*; Cobas Amplicor Roche

# TaT

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

## 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.

**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

# Clinical Impact of DAT

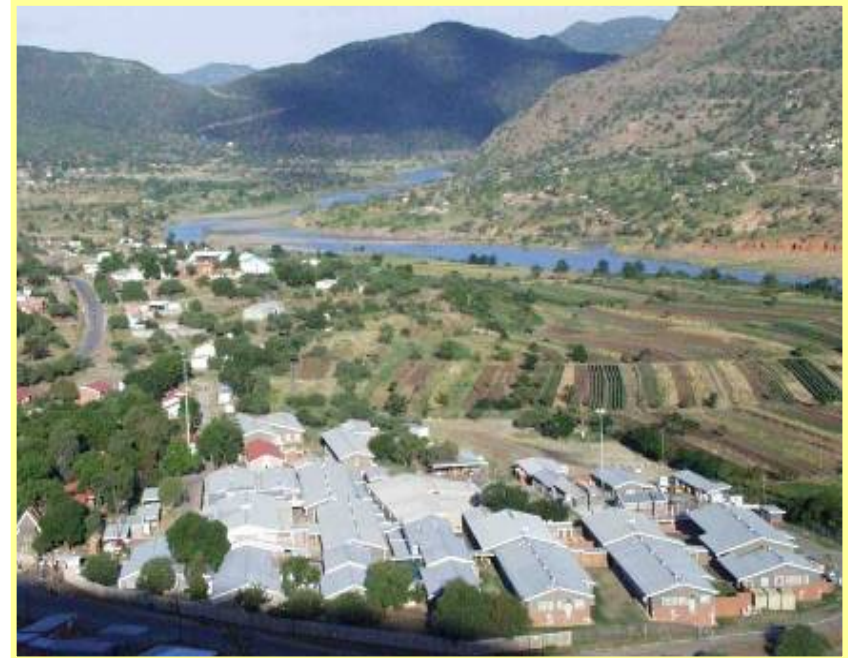
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Clinical Suspicion	Microsc	TB diagnosis	Clinical Impact DATs
H	Positive	Likely	Limited
H	Negative	Possible	High
M	Positive	Unclear	High
Low	Negative	Unlikely	Low

# Testing for MDR: The South African warning

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- Msinga district: 1,539 TB, 542 C+, 221 MDR and 53 “possible” XDR
  - 52/53 XDR died (16 day median survival)
  - All 44 cases tested HIV +
  - 15 died in ARV treatment (2 staff)
  - 26 XDR (51%): new cases
  - **39/46 (85%) with genotyping had similar strains.**
- Gandhi NR, et al. Lancet 2006;368:1575



# Drug resistance in MTB complex

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- Natural resistance to antibiotics
  - hydrophobic cell envelope (permeability barrier);
  - drug efflux systems and drug-modifying enzymes
- Resistance is due to chromosomal point mutations leading to amino acid substitution
- Mutations occur spontaneously with different frequency for different drugs
- Resistance emergence is linked to a large bacterial population

# Rifampicin Resistance: the “gold target”

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- Key-drug in the anti-TB regimen
- Low rate of spontaneous mutations
- Mutations affect an hot-spot region in the *rpoB* gene

**Optimal candidate for the molecular detection of resistance to the drug**

# RIF resistance as surrogate marker for MDR TB

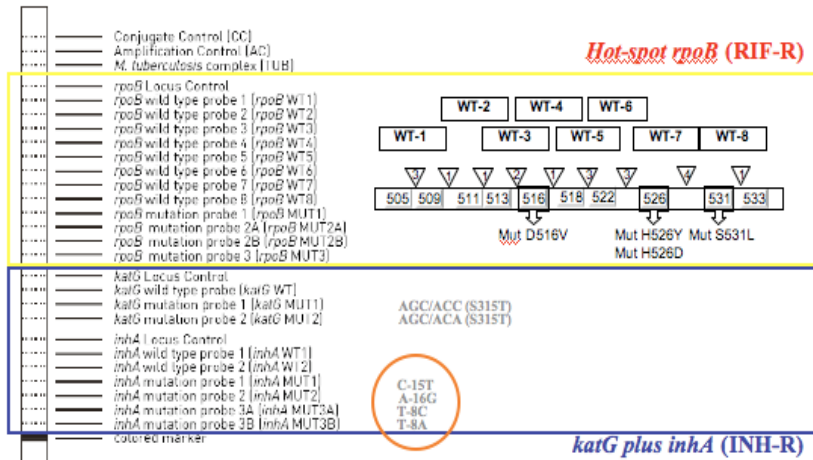
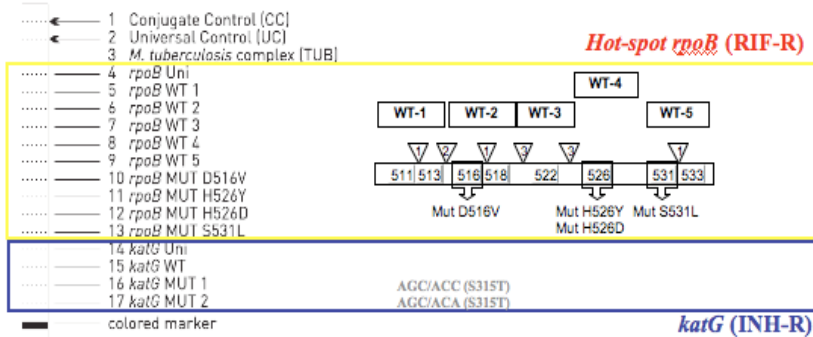
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- RIF resistance as a mono resistance not very frequent (5-15% of them)
- 80-95% of RIF resistant strains are also resistant to INH

<b>Line Probe Assay (LiPA)</b>	<b>Easy to perform and easy read-out; cost-effective</b>	<b>Limited number of probes that can be used;  Fails to distinguish insertions mutations</b>	<b>InnoLipaRif-TB GenotypeMTB-DRplus/si</b>
<b>Microarray</b>	<b>High throughput for screening due to the high number of probes;  High automation (high standardization)</b>	<b>Higher number of probes → higher complexity in results interpretation;  reproducibility of data;  Cost-effectiveness? To be evaluated</b>	<b>Several under development</b>
<b>Real-time PCR</b>	<b>Fast, totally automated</b>	<b>Expensive, few probes</b>	<b>Gene-Xpert</b>

# Commercial Line Probe Assays

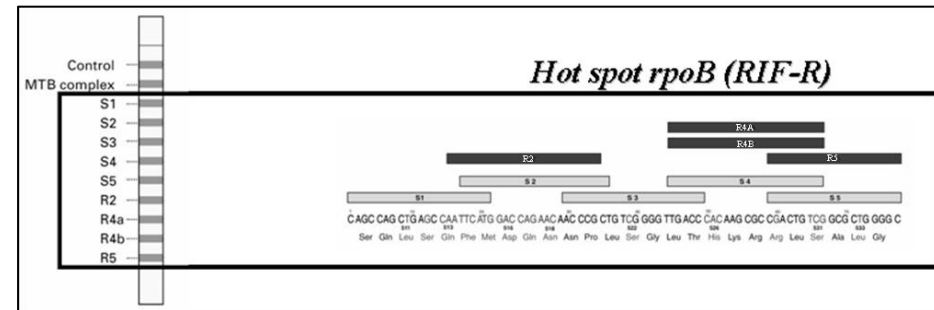
## Hain Lifescience



## Innogenetics

### INNO-LiPA-Rif.TB

Genotype  
MTBDR



Genotype  
MTBDRplus

## Comparison GenoType® MTBDR and INNO-LiPA Rif.TB

	GenoType® MTBDR	INNO-LiPA Rif.TB
<b>Company</b>	<b>Hain Lifescience</b>	<b>Innogenetics</b>
<b><i>M. tuberculosis</i> detection</b>	<b>Yes</b>	<b>Yes</b>
<b>Detection of RMP Resistance in <i>M. tb</i> Complex</b>	<b>Yes</b>	<b>Yes</b>
<b>Detection INH Resistance in <i>M. tb</i> Complex</b>	<b>Yes</b>	<b>No</b>
<b>Strip Assay</b>	<b>Yes</b>	<b>Yes</b>
<b>DNA-Basis: PCR</b>	<b>Yes</b>	<b>Yes</b>
<b>Culture requested</b>	<b>Yes</b>	<b>Yes</b>
<b>Direct assay</b>	<b>No</b>	<b>Yes (modified version)</b>
<b>M. tub-Komplex Detection: 23S-rRNA/16S-rRNA</b>	<b>Yes</b>	<b>Yes</b>
<b>RMP-Resistance: rpoB gene</b>	<b>Yes</b>	<b>Yes</b>
<b>INH-Resistance: katG gene</b>	<b>Yes</b>	<b>No</b>
<b>Universalcontrol</b>	<b>Yes</b>	<b>No</b>
<b>rpoB unicontrol</b>	<b>Yes</b>	<b>No</b>
<b>kat G unicontrol</b>	<b>Yes</b>	<b>No</b>

# Molecular DST in isoniazid-resistance detection: challenges

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1: [J Antimicrob Chemother.](#) 2009 Jan;63(1):11-6. Epub 2008 Oct 21.

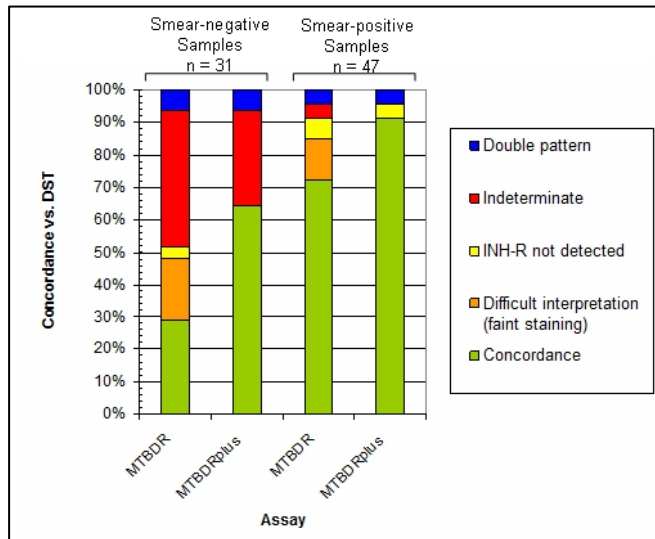
**Rapid genotypic assays to identify drug-resistant *Mycobacterium tuberculosis* in South Africa.**

[Evans J](#), [Stead MC](#), [Nicol MP](#), [Segal H](#).

Division of Medical Microbiology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa.

**OBJECTIVES:** Molecular assays to detect drug resistance in *Mycobacterium tuberculosis* are more rapid than standard drug susceptibility testing. To evaluate the efficacy of such assays in this setting, the GenoType MTBDRplus assay (HAIN Lifescience) and multiplex allele-specific PCR assays were carried out. **METHODS:** The GenoType MTBDRplus assay was evaluated for the detection of rifampicin and isoniazid resistance in 223 *M. tuberculosis* isolates of known phenotypic drug sensitivity. The presence of KatG S315T and inhA C-15T mutations that confer isoniazid resistance was determined using multiplex allele-specific PCR assays. The relationship between isolate lineage and resistance determinant was investigated by spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat analysis. **RESULTS:** The GenoType MTBDRplus assay detected multidrug-resistant, isoniazid-monoresistant and rifampicin-monoresistant isolates with sensitivities of 91.5%, 56.1% and 70%, respectively. Multiplex allele-specific PCR detected isoniazid resistance in 91.5% of the MDR isolates and 53.7% of the isoniazid-monoresistant isolates. The W-Beijing lineage was overrepresented in the MDR subgroup of strains (odds ratio 3.29; 95% confidence interval 1.76-6.16). **CONCLUSIONS:** A proportion of isoniazid resistance, particularly in isoniazid-monoresistant isolates of lineage X3, is due to resistance determinants other than KatG S315T and inhA C-15T. The fact that these isolates will be indicated as drug susceptible highlights the need for determining local patterns of resistance mutations to provide users with information regarding the capabilities of rapid genotypic assays.

# Sensitivity and Specificity



Miotto et al JCM 2008

**TABLE 5** Pooled summary estimates for rifampicin resistance

Subgroup	Pooled sensitivity	Pooled specificity
All rifampicin studies <sup>#</sup>	98.1 (95.9–99.1)	98.7 (97.3–99.4)
Only MTBDRplus assays <sup>¶</sup>	98.4 (95.1–99.5)	98.9 (96.8–99.7)
Only clinical specimens <sup>+</sup>	98.6 (95.5–99.6)	98.5 (96.9–99.3)

**TABLE 7** Pooled summary estimates for isoniazid resistance

Subgroup	Pooled sensitivity	Pooled specificity
All isoniazid studies <sup>#</sup>	84.3 (76.6–89.8)	99.5 (97.5–99.9)
Only MTBDRplus assays <sup>¶</sup>	88.7 (82.4–92.8)	99.2 (95.4–99.8)
Only clinical specimens <sup>+</sup>	84.5 (72.1–92.0)	99.2 (96.4–99.8)

DI Ling et al 2008. ERJ 32:1165-1174

# Molecular line probe assays for rapid screening of patients at risk of MDR-TB

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## **Policy statement by WHO and Partners**

**June 27, 2008**

Endorsement of the two commercial line probe assays for rifampicin resistance detection:  
Tests are CE marked and meet predefined performance targets in controlled evaluation studies

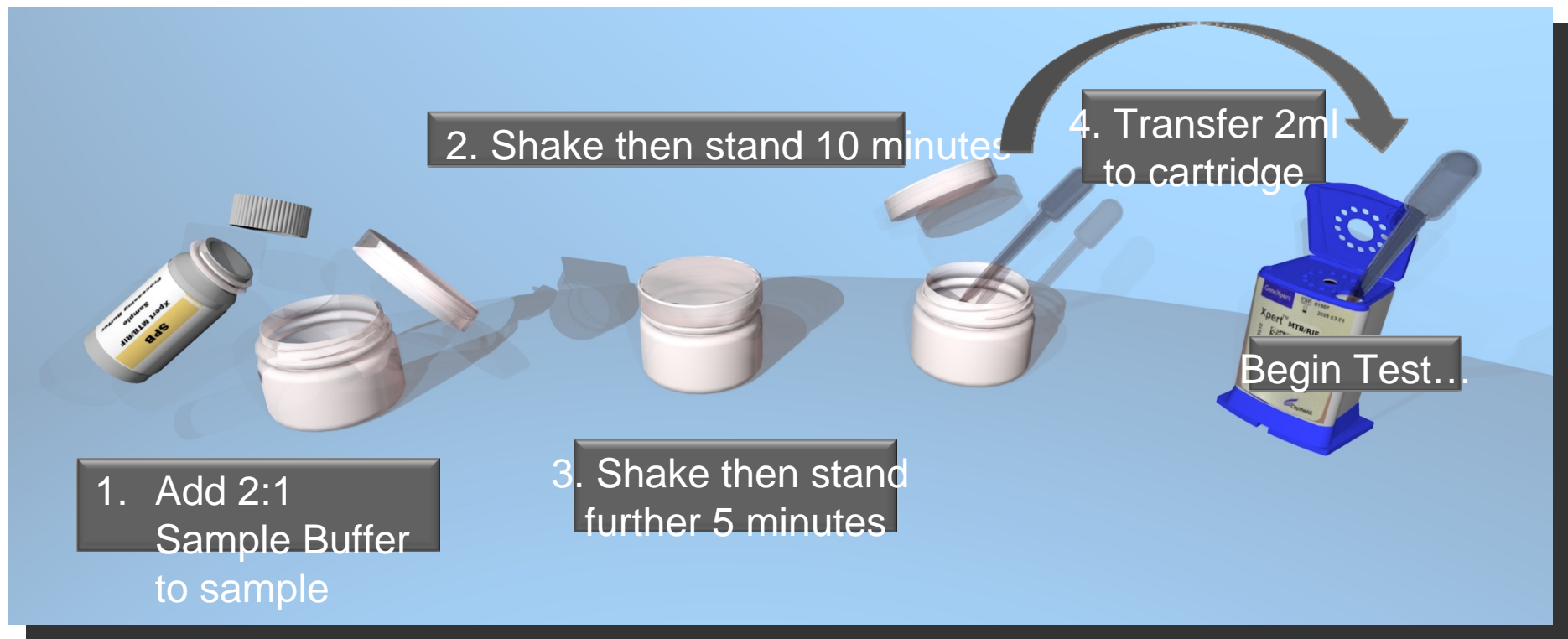
Both tests are highly sensitive and specific for rifampicin resistance detection from TB strains

# Xpert MTB/RIF Analytical Studies

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- Analytical Sensitivity of approximately approx 150 cfu/ml (Smear 10,000 cfu/ml)
- Specificity tested with high concentrations of MOTT (Mycobacteria Other Than Tuberculosis)
- No evidence of amplicon cross-contamination
- Perfect score on QCMD TB Proficiency Panel
- Mutation detection capability confirmed with isolate DNA and artificial targets having a global frequency reported at  $> 0.005$

# Simple Sample Processing – Direct Sputum



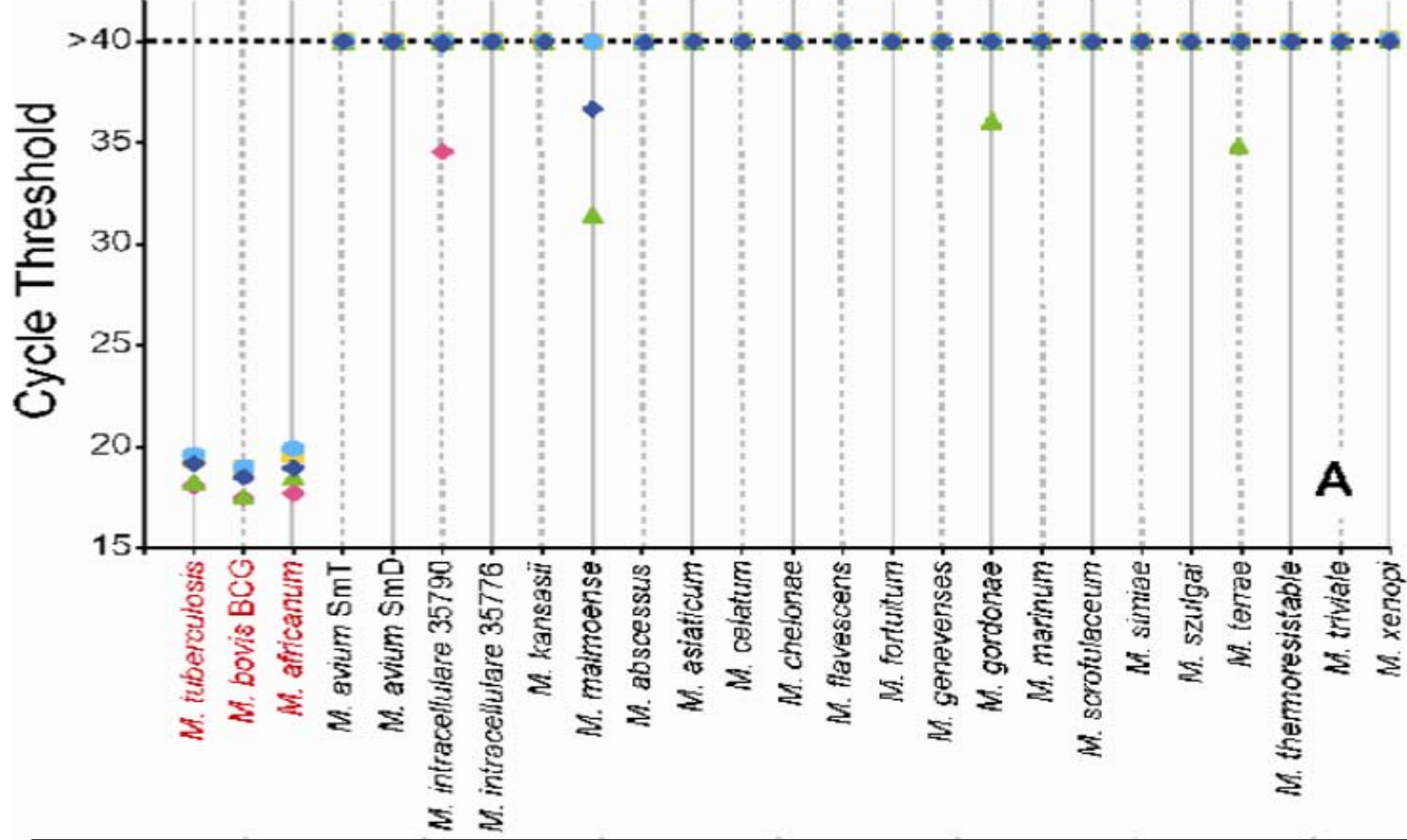
# Sensitivity and Specificity

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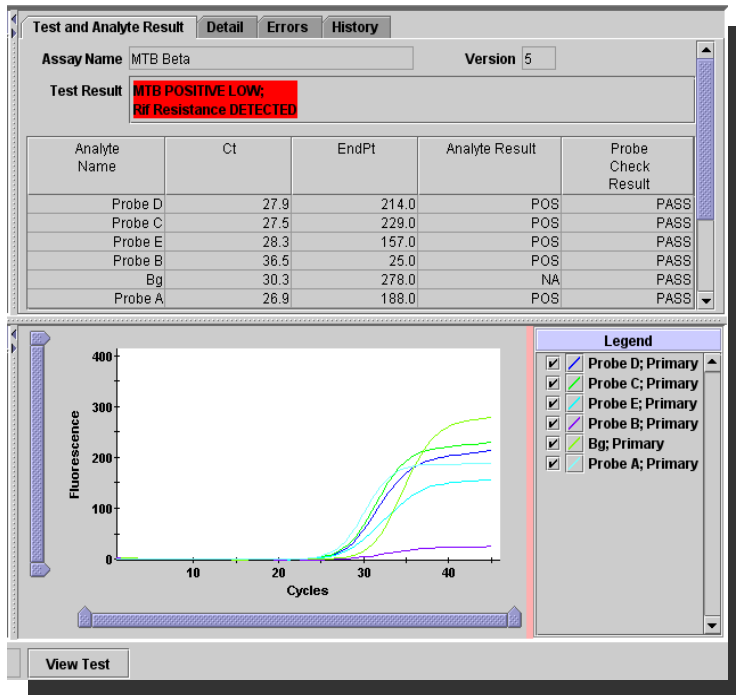
	Smear negative		Smear Positive
	Culture Positive	Culture Negative	Culture Positive
Xpert Positive	70	5	275
Xpert Negative	7	289	0

- Sensitivity in smear negative, culture positive (S-C+) was 90.9% (70/77)
- Sensitivity in smear positive, culture positive (S+C+). was 100% (275/275)
- Specificity of the assay was 98.3%
- Sensitivity observed for Rifampicin resistance was 96.7%
- Specificity observed for Rifampicin resistance was 98.6%

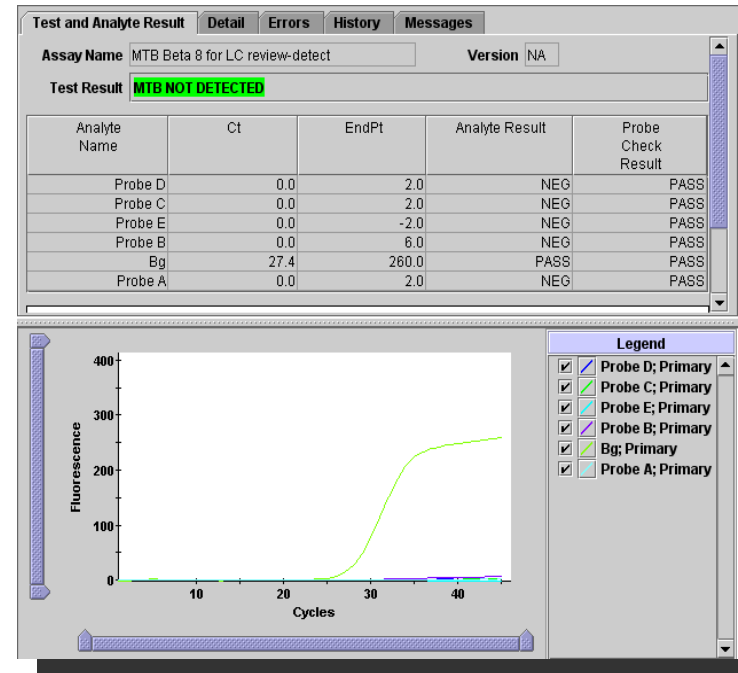
# MOTTs and Xpert MTB/RIF



# Amplification plots

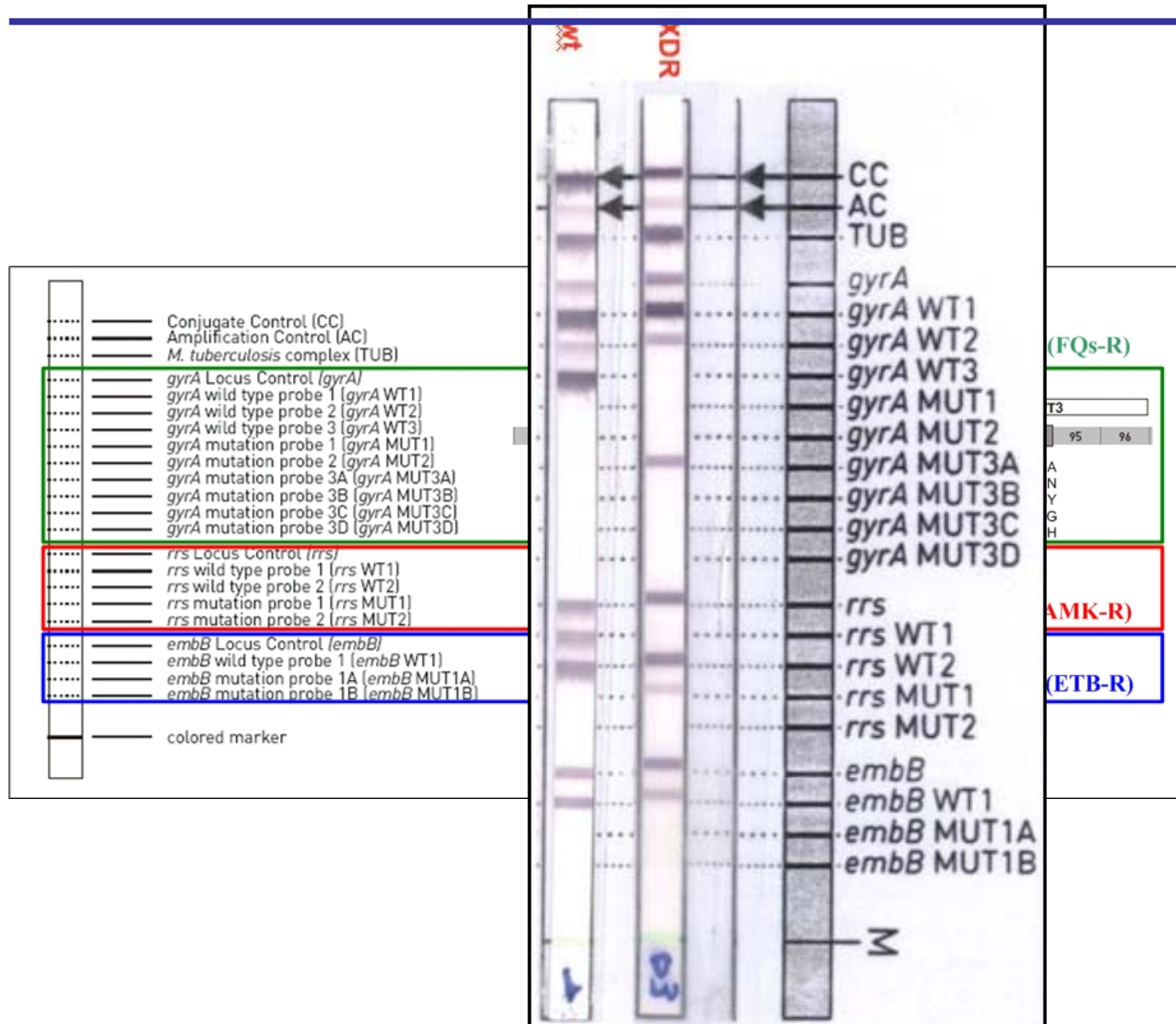


MTB Positive Low, Rif Resistance DETECTED



MTB not detected

# GenoType MTBDR<sub>sl</sub> (Hain Lifescience)



# Cost- related issues

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- Commercial tests are expensive:
  - Cost containing strategies ( centralizing/ diagnostic algorithms,..)
- May become cost effective if :
  - Increasing diagnostic accuracy
  - Reducing hospital admission costs

# Comparison of different strategies of DATs

JCM 2008; 46: 3811-3812

**E-MTD**

Testing strategy	Cost (\$) per PTB suspect diagnosis	% probability of correct diagnosis
CDC guidelines	68.3	0.91
Simultaneous testing	102.7	0.96
Smear-positive dilution	53.4	0.94
Sequential dilution	90.9	0.96

# Considerations on cost-effectiveness of molecular DST

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- *Cost of laboratory facilities*
- *Cost of trained personnel*
- *Treatment options should be available*
- *Containment strategies should be available*
- *Saving on new infection's cost*

**A country tailored diagnostic algorithm should be developed considering:**

- **Rate of MDR (reliable data:DRS performed, QA in place)**
- **Population at risk and/or subgroups**

# Conclusions

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- Clear algorithms for introduction of DATs should be developed considering:
  - Local epidemiological data
  - Suboptimal sensitivity on sm- and EP samples
  - Cost
  - Patients admission policies/ contact tracing policies
  - Availability of fast TaT
- Choice of methodology could be based on technical capacity or other molecular routine in the laboratory. Appropriate biosafety level should be in place
- Mol DST 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.

# Molecular DST: conclusions

- ***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.***
- ***Usefulness of molecular techniques in DST limited by the lacks of knowledge of all molecular mechanisms of resistance.***
- ***Appropriate EQAs and training packages needs to be developed***
- ***Identification of specific mutations allows to predict cross-resistances among drugs (e.g. aminoglycosides).***
- ***Molecular test may be easier to be implemented than liquid cultures and could substitute conventional DRS for collection of MDR data***

# EQA

Noordhoek et al. CMI 2004;10: 295-301

Panel samples	Amplicor	LCx	E-MTD	Probe Tec	Top score available
Sputum mean score	14.7	16.0	13.6	12.8	16
PBS mean score	4.6	3.3	5.3	2.8	6
Total	19.3	19.3	18.9	15.6	22

High intercenters variability

High false negative among sm-(38.8%)

4% false positive

# EQA QCMD/INSTAND

Year	2001	2002	2003	2004	2005	2006
Score	8	24	20	20	24	20
COBAS Roche	6.7	20.1	16.4	19.0	-	-
E-MTD	5.3	16.1	16.1	17.2	19.3	17.6
B-D ProbeTec	4.8	19.5	15.7	17.7	18.5	16.6
RT Real Art	-	-	-	18.2	20.2	18.4

Method	Mean success rates (%)				
	2002	2003	2004	2005	2006
COBAS Amplicor	98	91	99	98	89
Gen-Probe E-MTD	92	94	100	87	94
Probe Tec	97	98	99	98	97
In-house PCR	98	92	98	97	93