

# **Bacteriological and Molecular Diagnosis of Childhood TB in Low / Intermediate Burden Settings**

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# **Microbiological Diagnostic Tools**

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**Microscopy**

**Culture**

**PCR**

**Array technique (chip)**

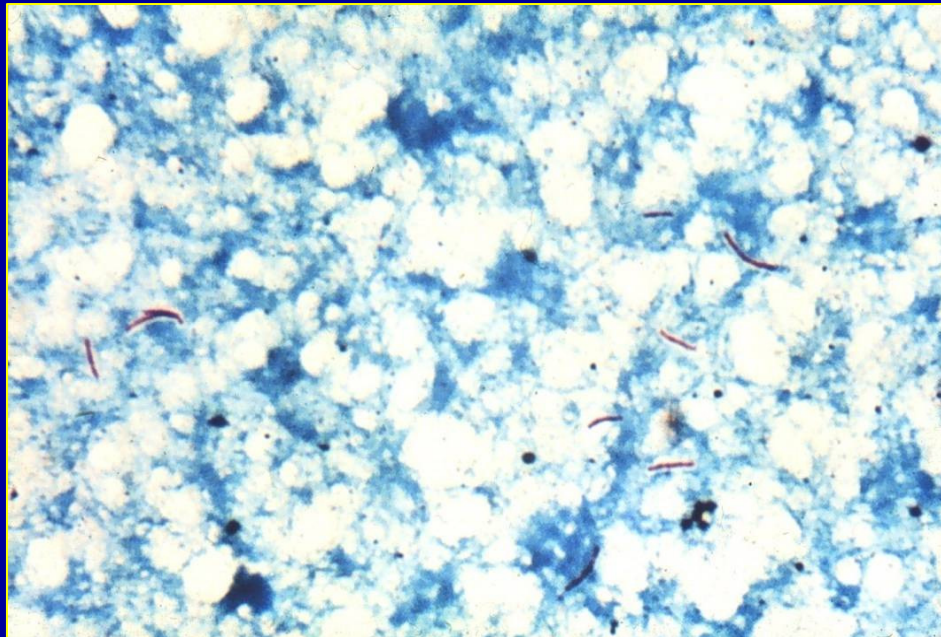
# Microbiological Diagnostics

## Ziehl Neelsen Staining/Fluorescence microscopy

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Detection limit:	$10^4$ bacteria/ml
Turn-around time:	20 min
Test sensitivity:	~ 20% (~30%*)

*\*fluorescence microscopy*



*Lighter J, Rigaud M*

*Diagnosing childhood tuberculosis: traditional and innovative modalities.  
Curr Probl Pediatr Adolesc Health Care 2009 39: 61-88*

# Microbiological Diagnostics - Culture

**Gold standard (LJ) – high specificity**

Detection limit	10-100 bacteria/ml
Turn-around time:	liquid medium (~14 days) solid medium (~ 4 weeks)
Sensitivity:	liquid ~ 50% solid ~ 30-40%



*Lighter J, Rigaud M  
Diagnosing childhood tuberculosis: traditional and innovative modalities  
Curr Probl Pediatr Adolesc Health Care 2009 39: 61-88*

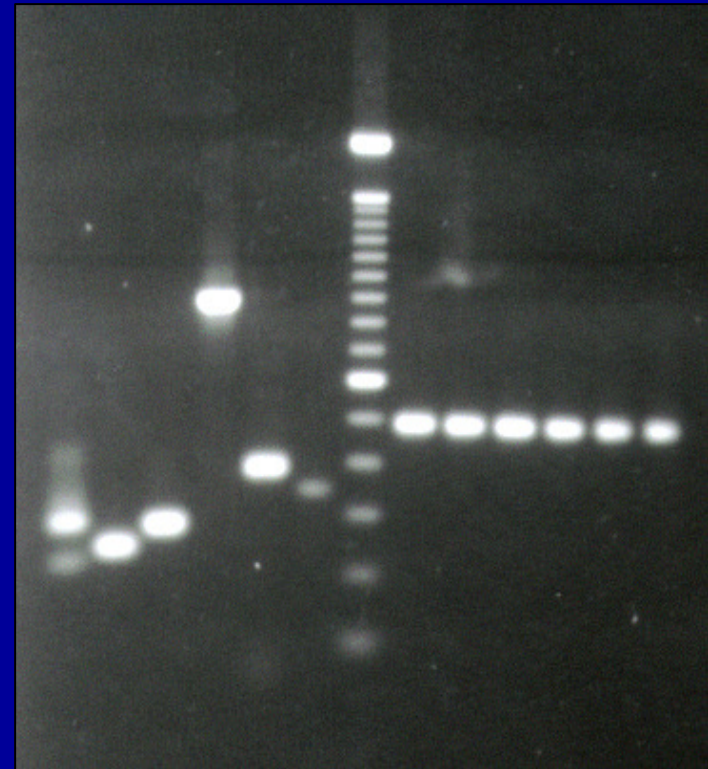
# Microbiological Diagnostics

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**Detection limit:** ~ 10 bacilli  
**Turn-around time:** ~ 24-48 h  
**Sensitivity:** 40-60%  
in smear negative  
but culture positive

90-100%  
in smear positive  
and culture positive

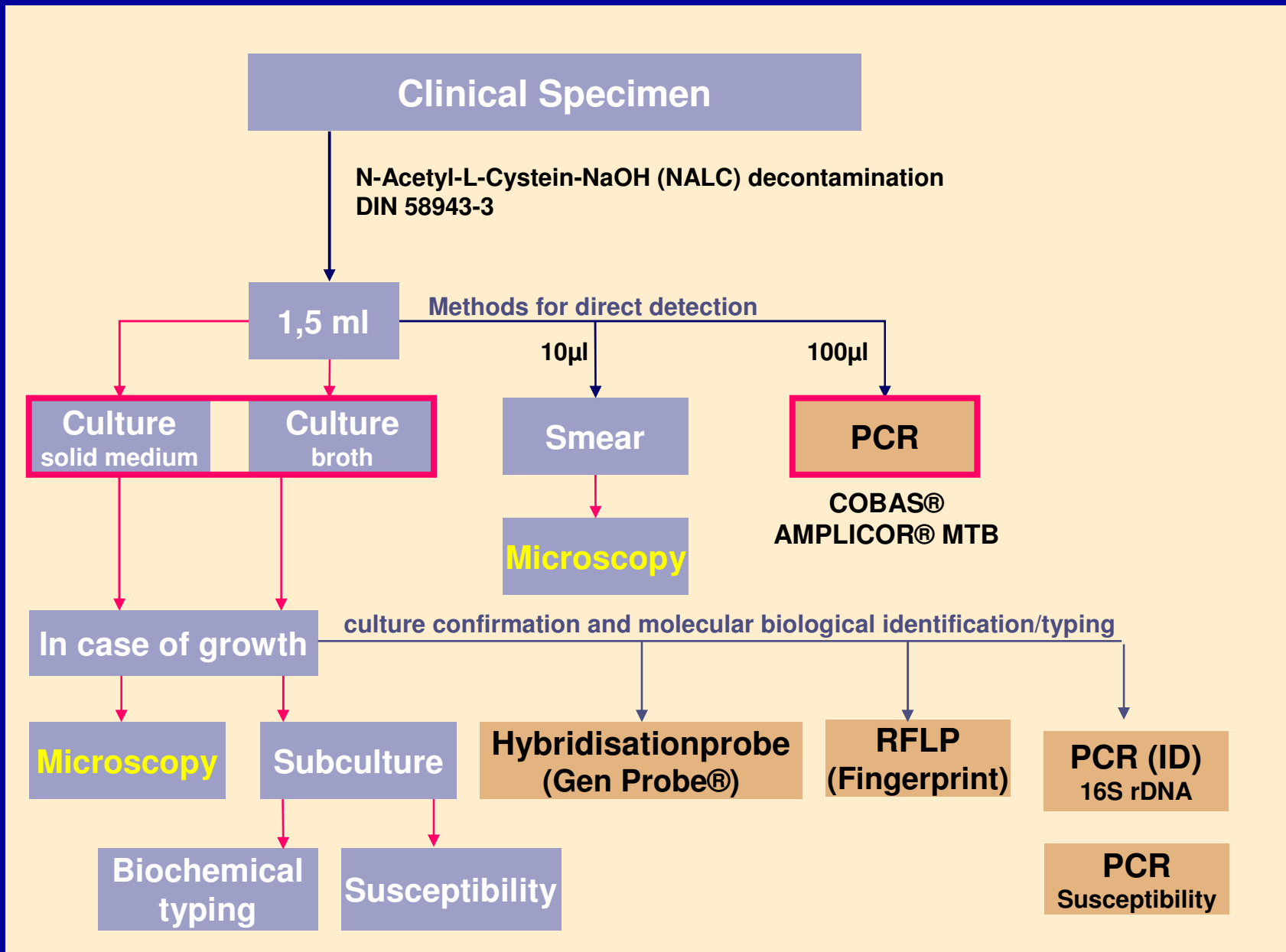
## PCR (NAAT\*)



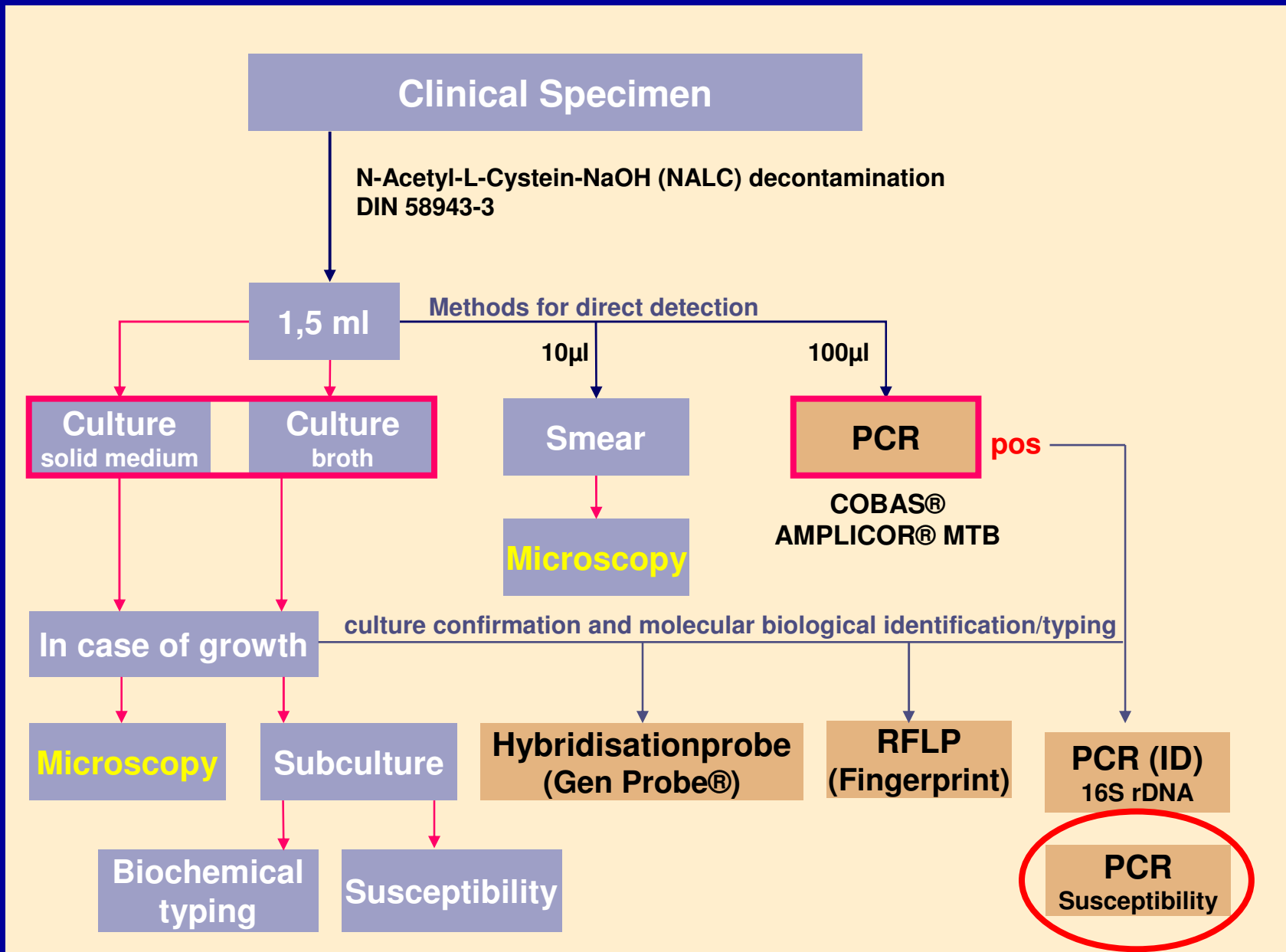
\*NAAT = Nucleic Acid Amplification Techniques

*Gomez-Pastrana D*  
*Tuberculosis in children-is PCR the diagnostic solution?*  
*Clin Microbiol Infect 2002 Sep 8:541-544*

# Bacteriological Workflow



# Bacteriological Workflow

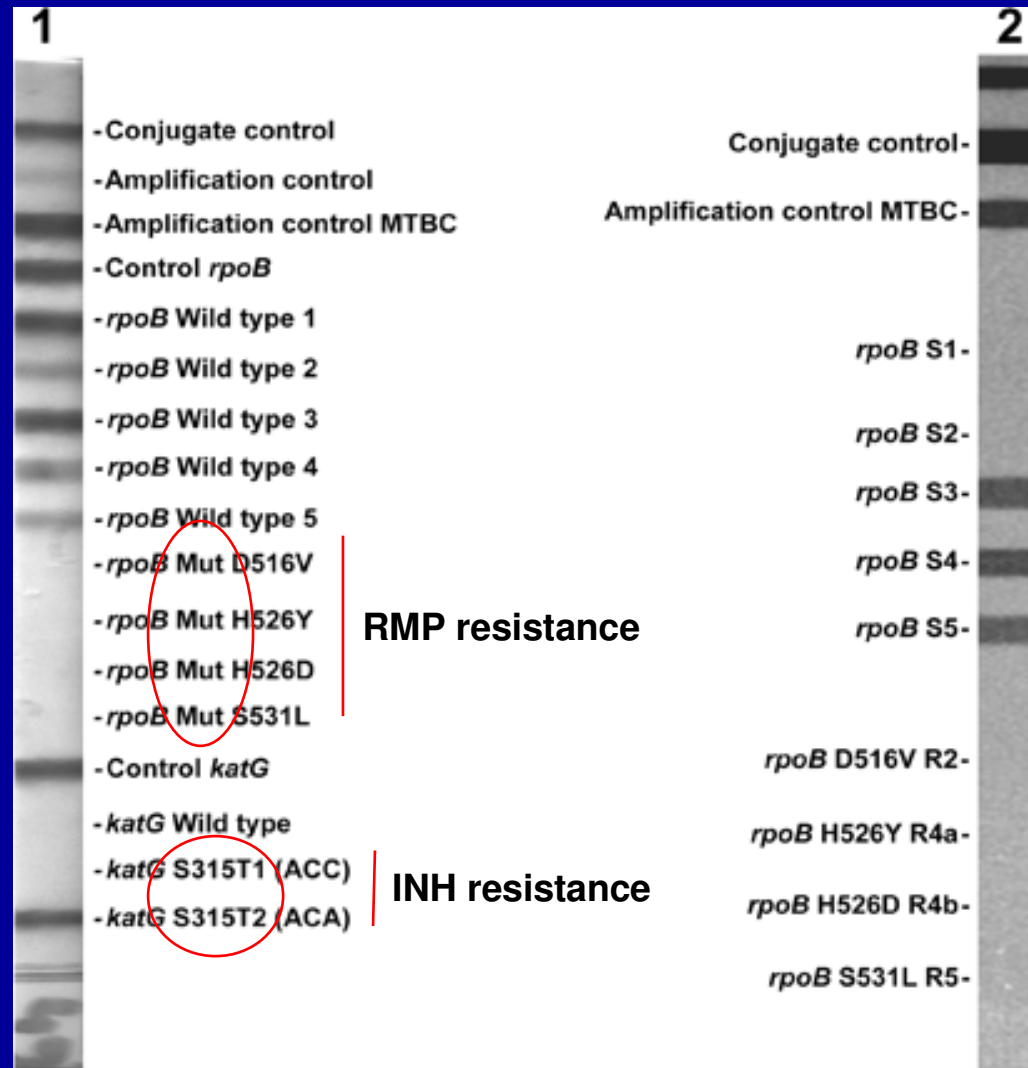


# PCR: Molecular susceptibility testing

## Lineprobe Assay

Hain  
Genotype  
MTBDR

- robust
- reliable
- low price
- labour intensive  
„washing“
- max. 18 samples



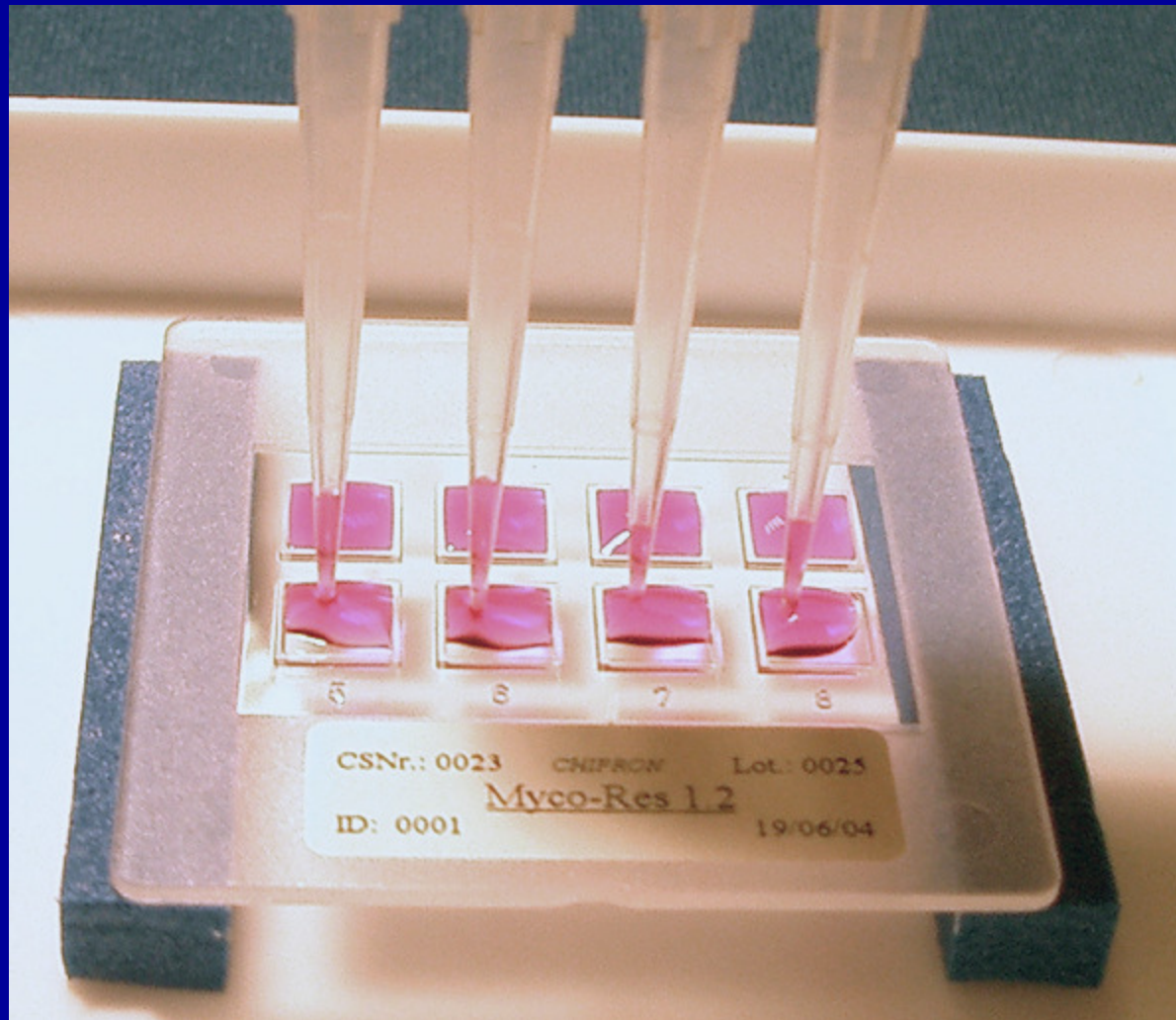
INNO-LiPA  
Rif.TB  
assay



**Array technique for  
identification and susceptibility testing  
(chip technology)**

# Chip (technology): low cost / low density array

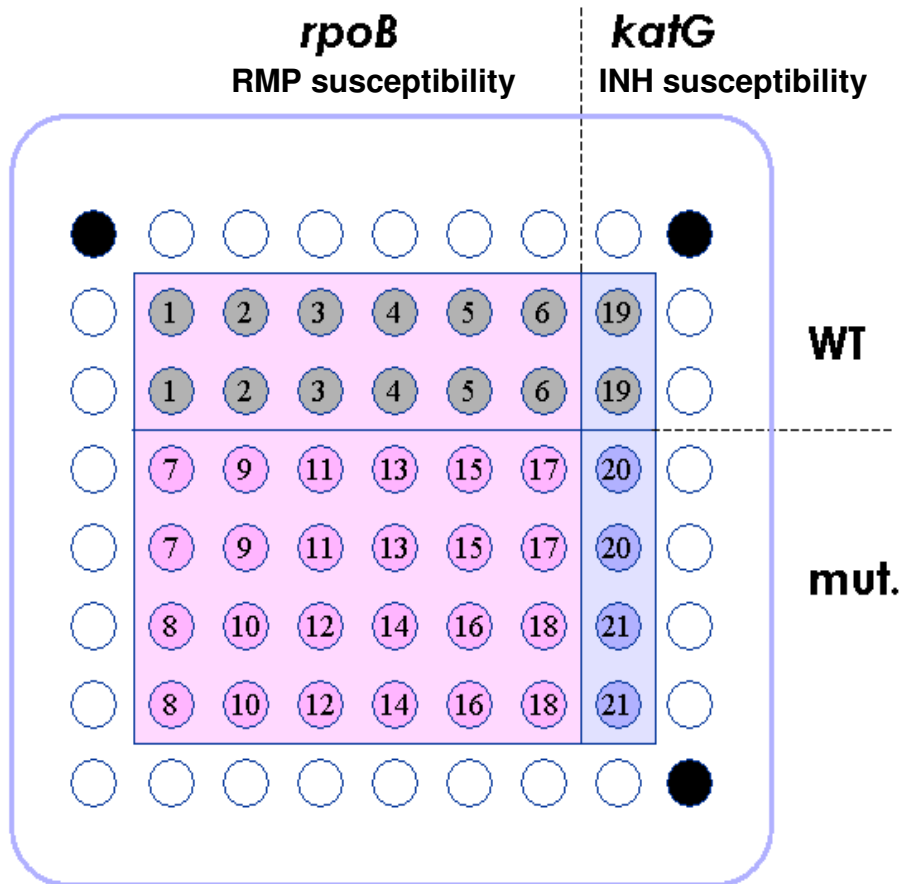
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# Suceptibility testing: Chip *Myco-Res*



Turn-around time: ~ 4 h



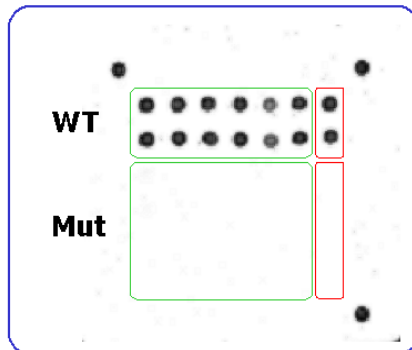
1	WT-AA-504-509	<i>rpoB</i>
2	WT-AA-509-515	<i>rpoB</i>
3	WT-AA-514-519	<i>rpoB</i>
4	WT-AA-520-524	<i>rpoB</i>
5	WT-AA-525-530	<i>rpoB</i>
6	WT-AA-530-534	<i>rpoB</i>
7	Mut-511-Pro	<i>rpoB</i>
8	Mut-512-Thr	<i>rpoB</i>
9	Mut-516-Tyr	<i>rpoB</i>
10	Mut-516-Val	<i>rpoB</i>
11	Mut-526-Asn	<i>rpoB</i>
12	Mut-526-Leu	<i>rpoB</i>
13	Mut-526-Asp	<i>rpoB</i>
14	Mut-526-Tyr	<i>rpoB</i>
15	Mut-526-Arg	<i>rpoB</i>
16	Mut-531-Leu	<i>rpoB</i>
17	Mut-531-Trp	<i>rpoB</i>
18	Mut-533-Pro	<i>rpoB</i>
19	WT-315-Ser	<i>katG</i>
20	Mut-315-Thr	<i>katG</i>
21	Mut-315-Asn	<i>katG</i>

# Suceptibility testing: Chip *Myco-Res*

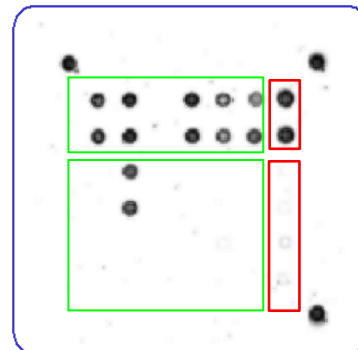


## Sputum (AFB +)

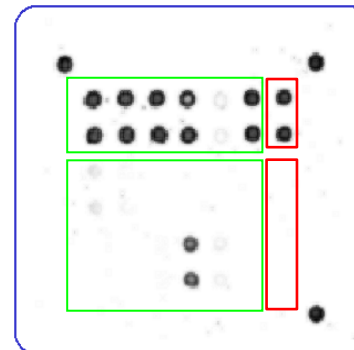
*rpoB*: **WT**  
*katG*: **WT**



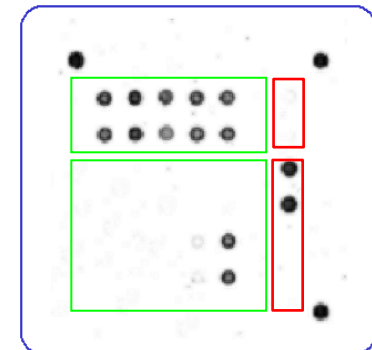
*rpoB*: **516-Tyr**  
*katG*: **WT**



*rpoB*: **526-Asp**  
*katG*: **WT**



*rpoB*: **531-Leu**  
*katG*: **315-Thr**



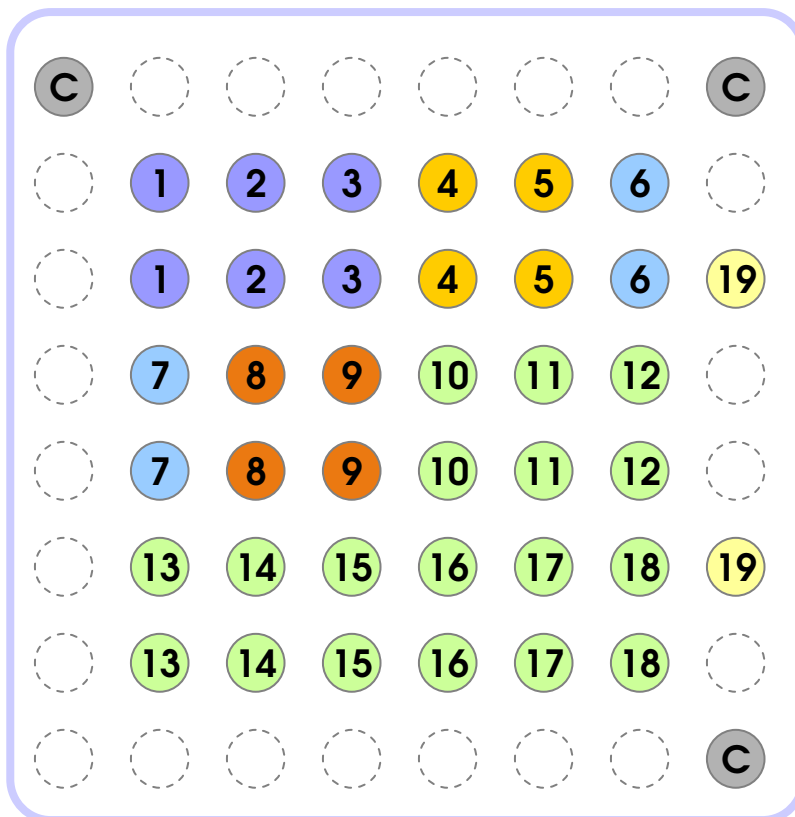
Capture probe for  
mutant 516-Tyr  
positive  
global: 3.9 %

Capture probe for  
mutant 531-Leu  
positive  
global: 43.2 %

# Identification: Chip *Myco-Direct 1.7*

Turn-around time: ~ max. 4 h

e.g. sputum (AFB +)



Nr.	ID	PCR Mix
1	Mycobacteria Group I	A
2	Mycobacteria Group II	A
3	Mycobacteria Group III	A
4	M. tub complex 01	A
5	M. tub complex 02	B
6	M. avium complex 01	A
7	M. avium complex 02	A
8	M. kansasii 01	A
9	M. kansasii 02	A
10	M. xenopi	A
11	M. abscessus	A
12	M. goodii	A
13	M. peregrinum	A
14	M. szulgai	A
15	M. haemophilum	A
16	M. marinum/ulcerans	A
17	M. simiae	A
18	M. smegmatis	A
19	Internal Standard	optional
C	Hyb-Control	-

**Clinical basic application**

**- Up to date recommendation -**

# CDC Updates Guidelines for Nucleic Acid Amplification Techniques to Diagnose Tuberculosis

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Laurie Barclay

*Morb Mortal Wkly Rep* 2009 58:7–10



- NAAT results should be interpreted in conjunction with the AFB smear results.
- NAAT and smear positive: start Rx despite pending culture results. PPV 95%
- Smear negative, NAAT positive: use clinical judgment to either treat or await culture

## Selection from automated systems for molecular and bacteriological rapid diagnostics

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### PCR:

Roche/COBAS<sup>®</sup>: Amplicor<sup>®</sup> amplification kits

Roche/COBAS<sup>®</sup>: LightCycler<sup>®</sup> (real-time-PCR)

Roche/COBAS<sup>®</sup>: TaqMan 48<sup>®</sup>

(increases the specificity of real-time-PCR)



### Culture:

BD Bactec<sup>™</sup> MGIT\* 960:

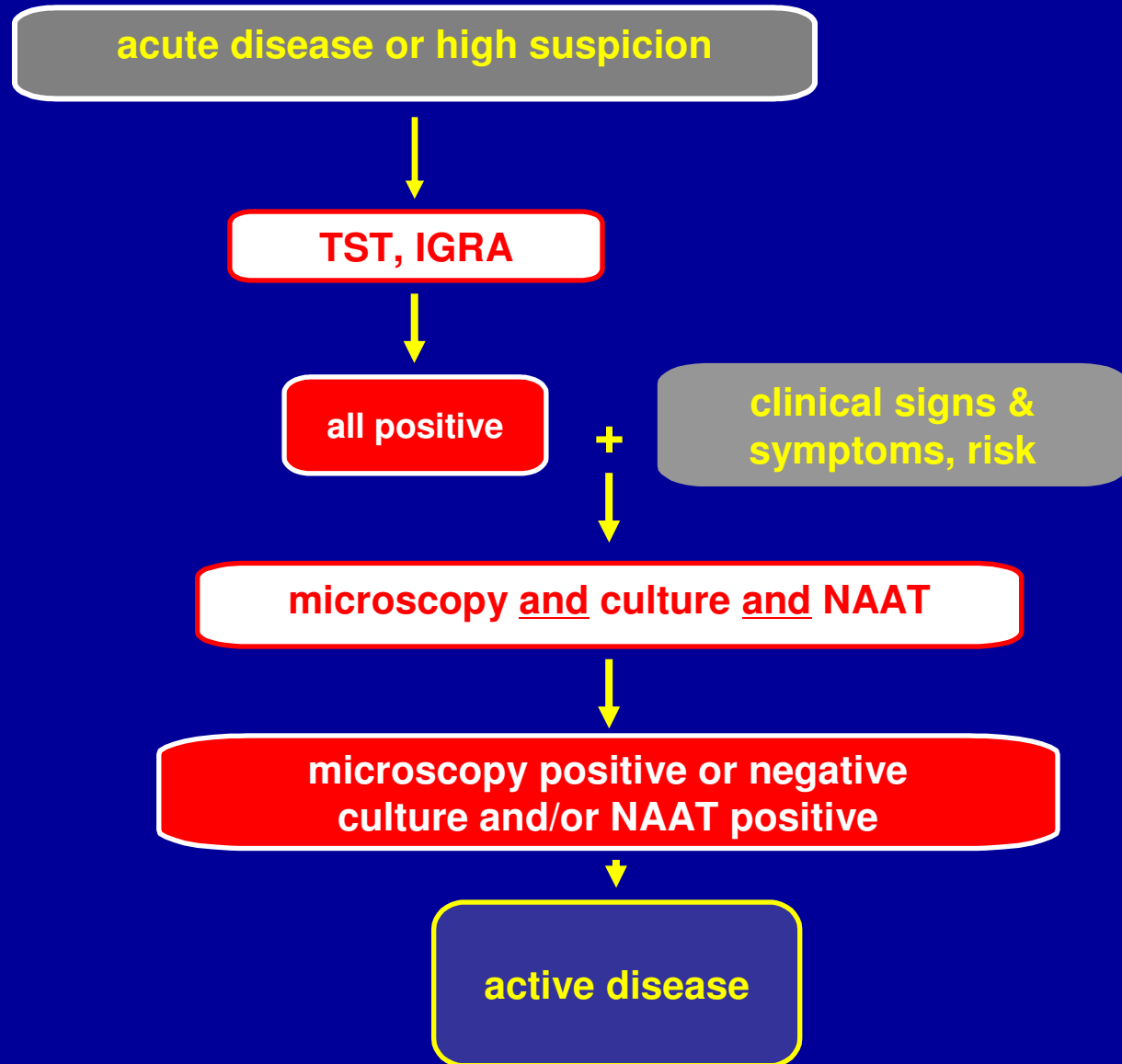
bacteriological broth diagnostics



\* **M**ycobacteria **G**rowth **I**nhibitor **T**ube



# Workflow: Diagnosis of Tb using microscopy, culture, and NAAT



# Workflow: Diagnosis of Tb using microscopy, culture, and NAAT

acute disease or high suspicion

TST, IGRA

all positive

clinical signs &  
symptoms, risk

microscopy and culture and NAAT

microscopy positive or negative  
culture and/or NAAT positive

active disease

## Why still microscopy?

- cheap
- easy
- fast
- semiquantitative
- specificity ?
- in combination with PCR  
100% specificity

# Summary

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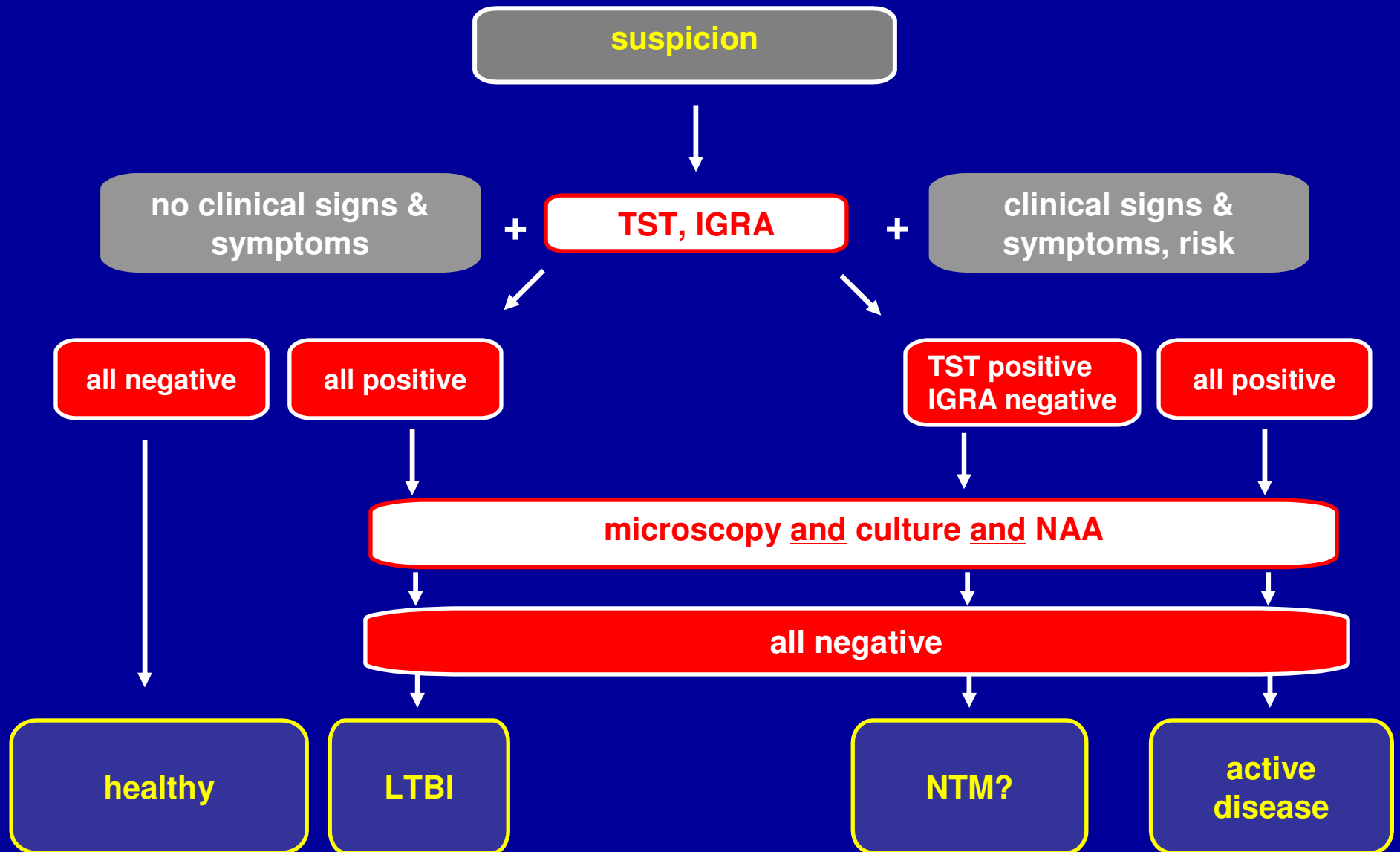
- 1. Principle methods for TB diagnostics are: microscopy, culture, and PCR**
- 2. PCR can't yet replace neither microscopy nor culture but it compliments both methods**
- 3. No testing method replaces clinical assessment**



# **Traditional and Modern TB-Diagnostics**

**Together they are strong**

# Workflow: Diagnosis of Tb using microscopy, culture, and NAAT





**Thank you for your attention!**



## **Rapid diagnostics ? ...**

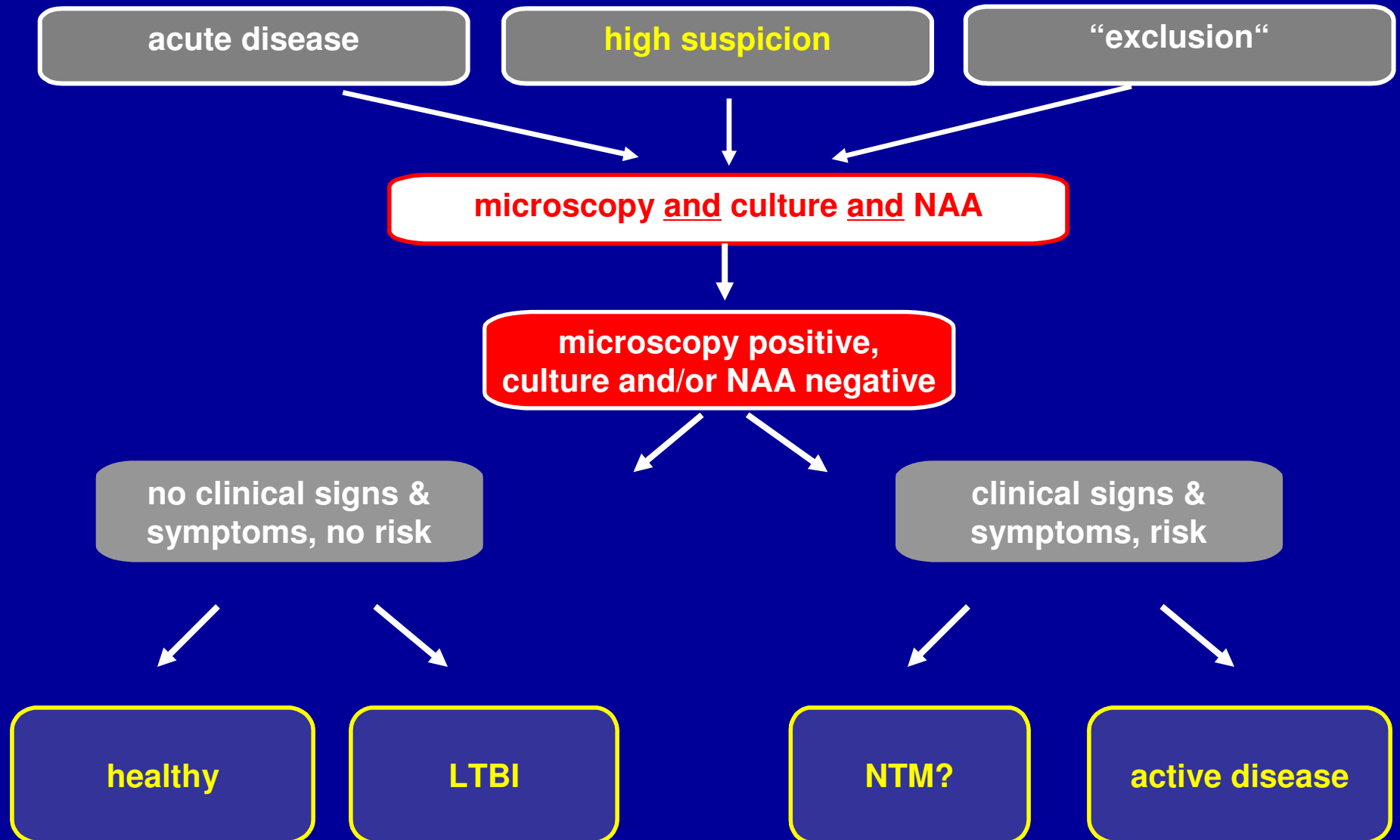
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**... save time and money  
(i.e. MDR direct detection)**

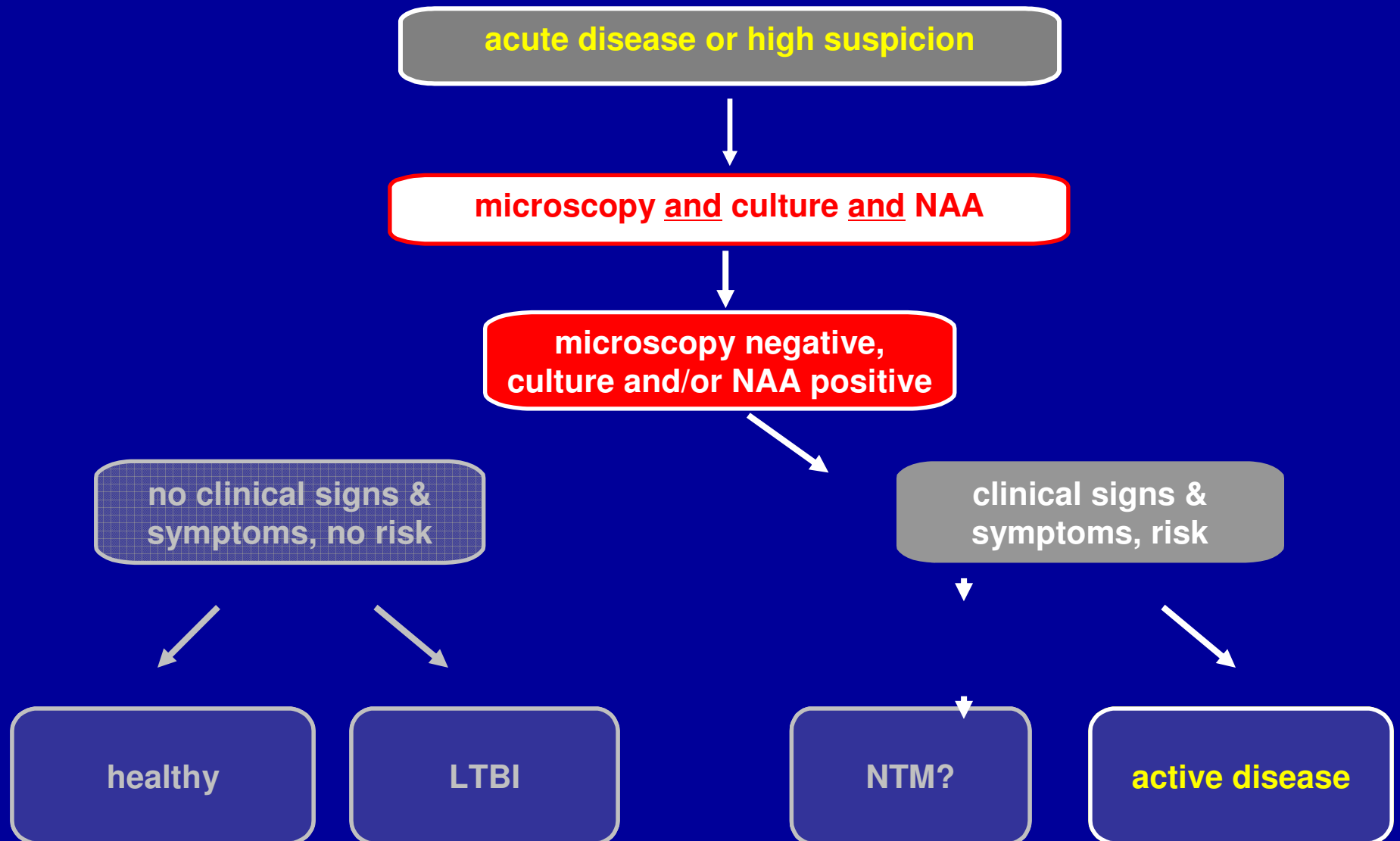
**... still no readiness to use  
so-called “expensive” PCR- tests**



# Workflow: Diagnosis of Tb using microscopy, culture, and NAA



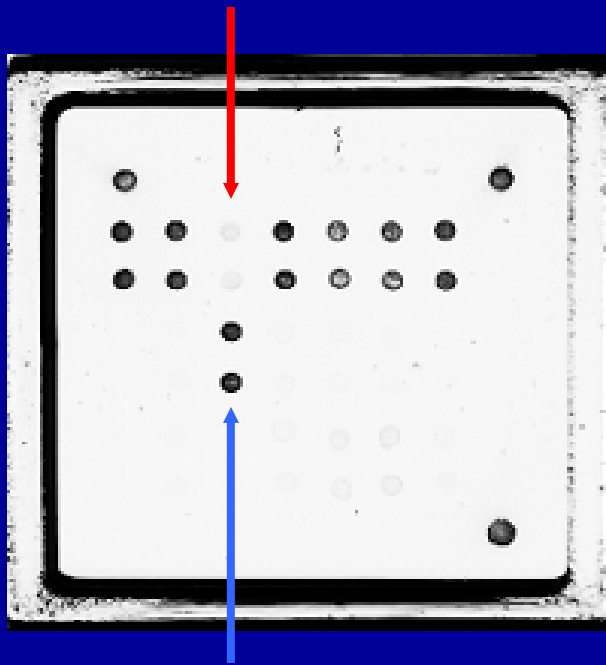
# Workflow: Diagnosis of Tb using microscopy, culture, and NAA



# LCD Array Myco-Res

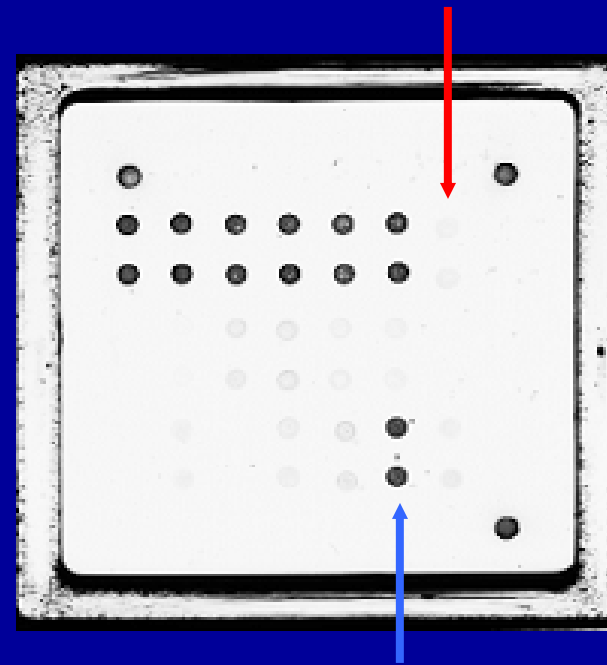


Capture probe  
WT amino acids 514-519



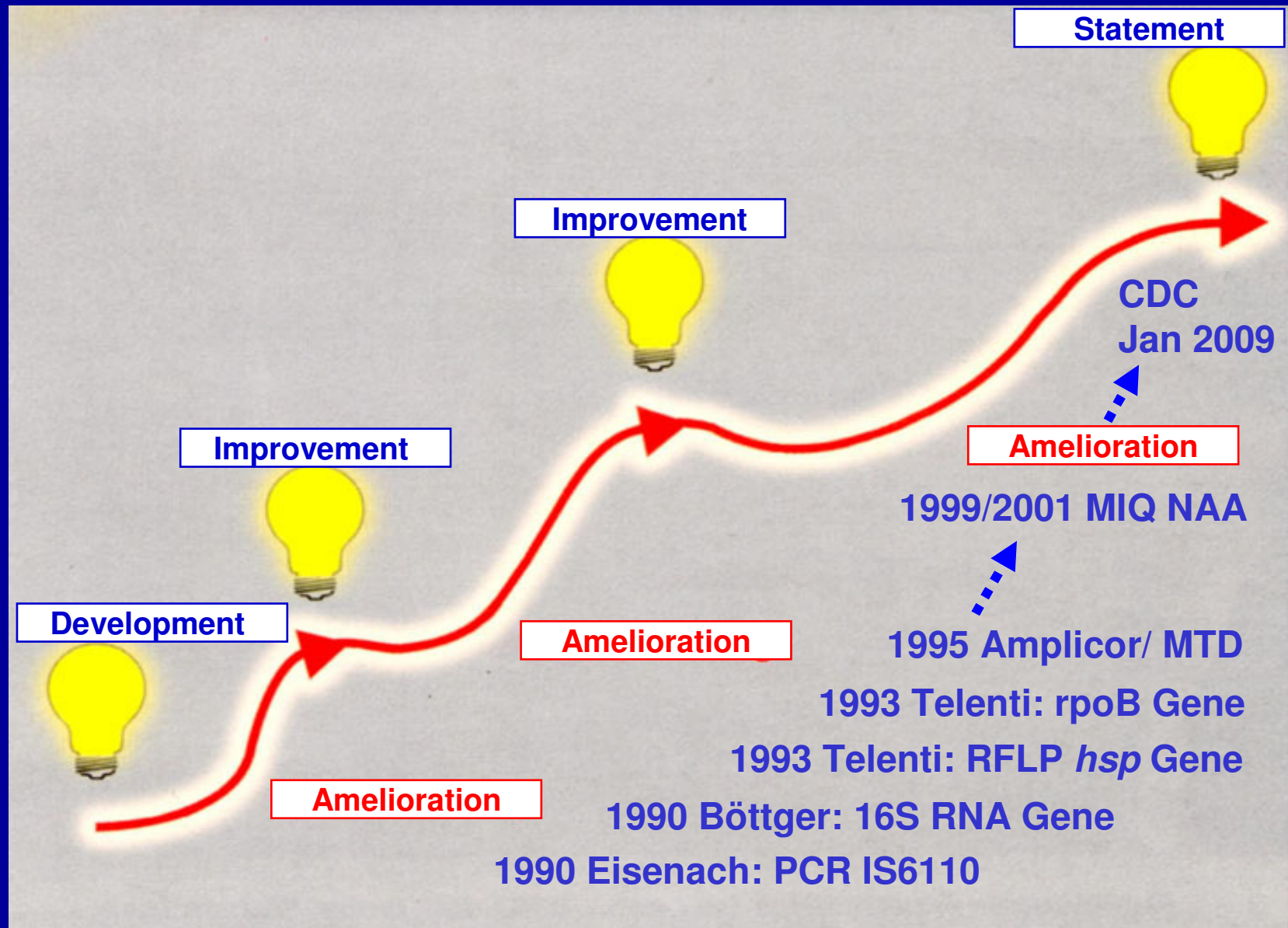
Capture probe for  
mutant 516-Tyr positive  
global: 3.9 %

Capture probe  
WT amino acids 530-534



Capture probe for  
mutant 531-Leu positive  
global: 43.2 %

# PCR Timeline

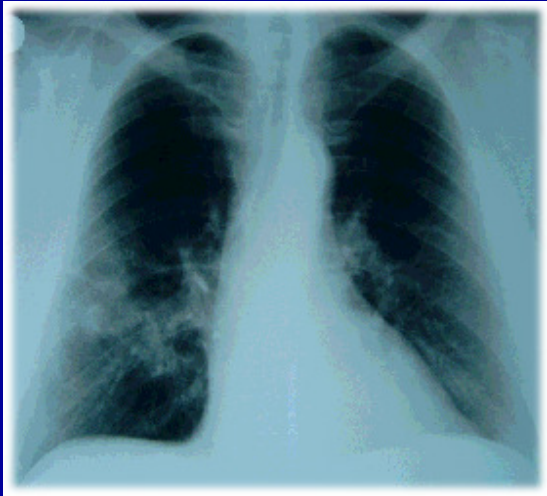


## **Capture PCR**

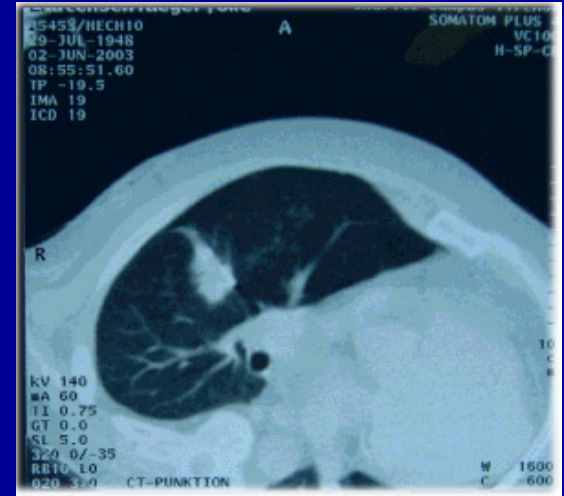
A PCR strategy in which linkers added to the ends of linear DNA molecules are used as primer binding sites and intramolecular stem-loop structures are exploited for strand specific priming. The products generated by amplification of the sequence between the two primers correspond can be captured by streptavidin.

## **Capture probe**

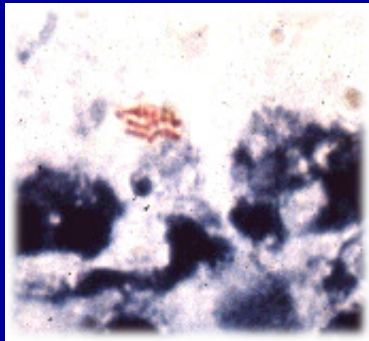
A phage or antibody probe that binds proteins in a sample enabling relative expression levels to be detected.



**infiltration/cavern  
abscess  
MDR-risk  
immunosuppression**



**probe**



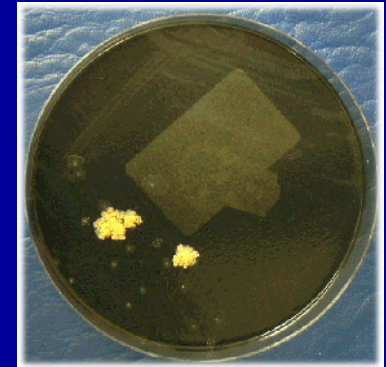
**SFS  
GPS  
mycel**

**PCR**

**MTB  
MOTT  
Noc  
RIF 90%  
INH 70%**



**culture sensitivity  
(phenotype)**



# Quality Control – Mycobacteria NAA - PCR Methods

Method	n	(% )	mean RQ	
			„simple“	vs all samples
Amplicor	54	46.6%	99 %	89 % (-10)
	57	58.8%	97 %	91 % (-5)
GenProbe	10	8.6%	100 %	94 % (-6)
	9	8.5%	98 %	94 % (-4)
In-house	28	24.1%	96 %	93 % (-3)
	21	19.8%	96 %	92 % (-4)
ProbeTec	20	17.2%	97 %	97 % (-0)
	14	13.2%	99 %	97 % (-2)
other	4	3.4%	100 %	82 % (-18)
	5	4.7%	88 %	83 % (-5)

**culture**



**sensitivity**

**LightCycler - Arrays**

**in house  
Amplicor**

**Genus PCR**

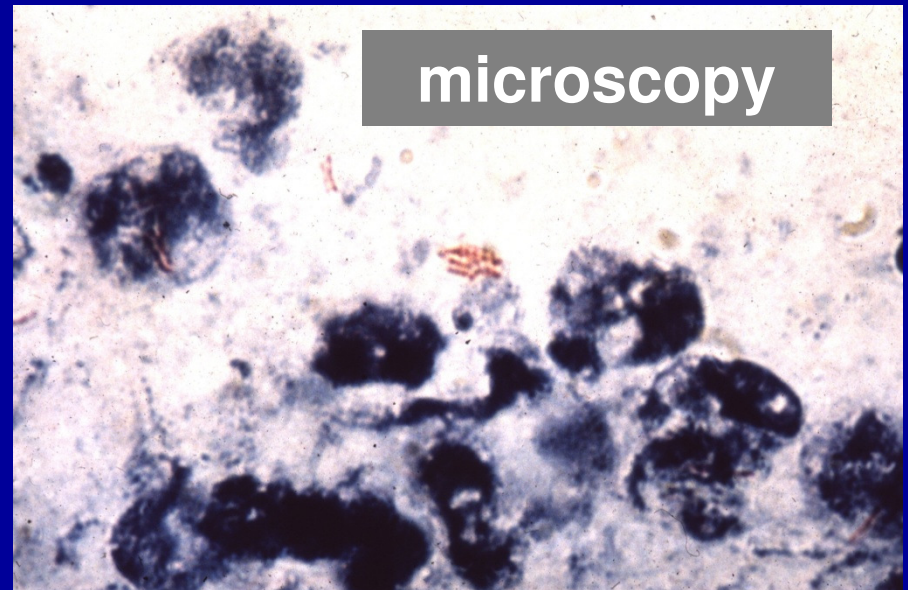
**TB PCR**

**chip technology**

**differentiation  
identification  
resistance**

**LightCycler - Arrays**

**microscopy**



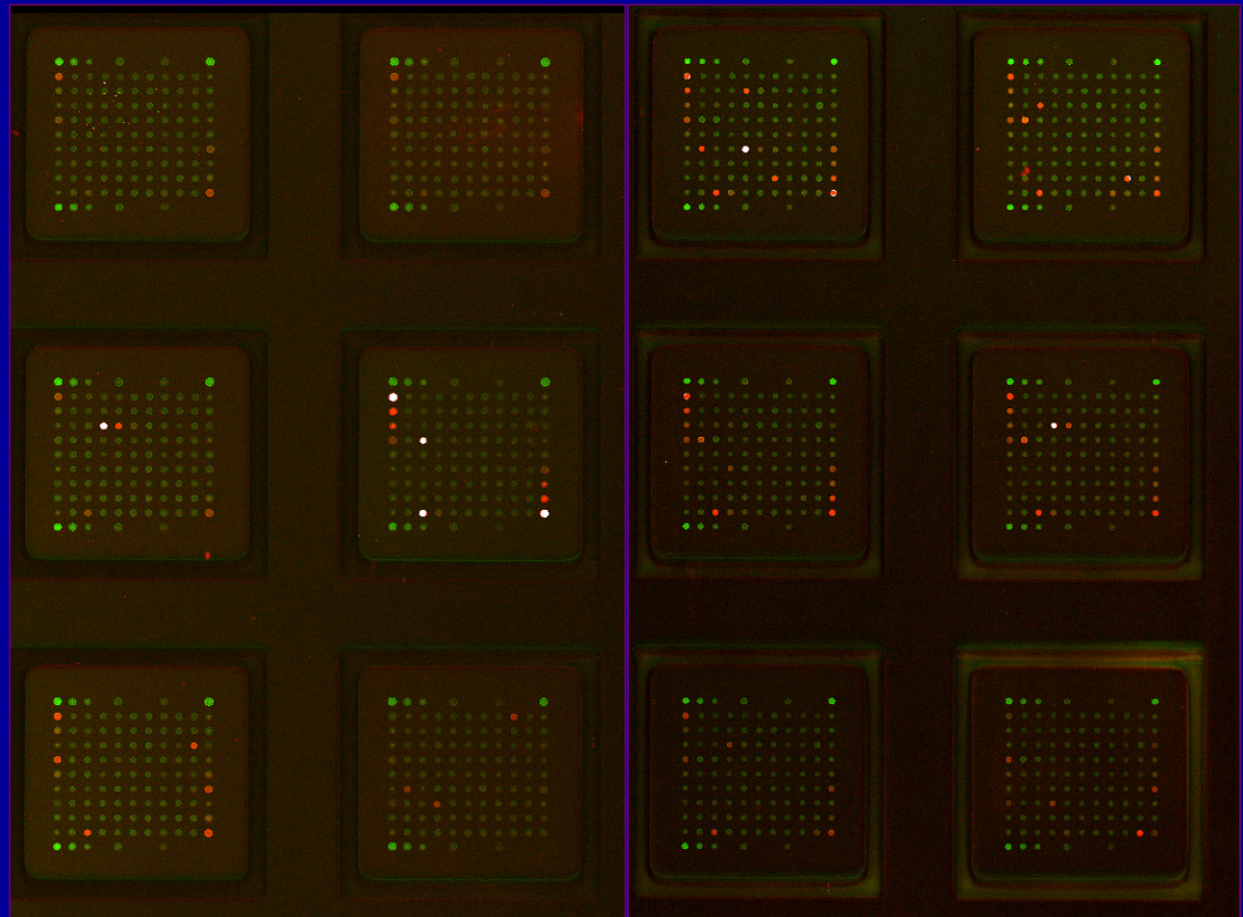


# Multigenotypic post-PCR analysis of multiple specificities

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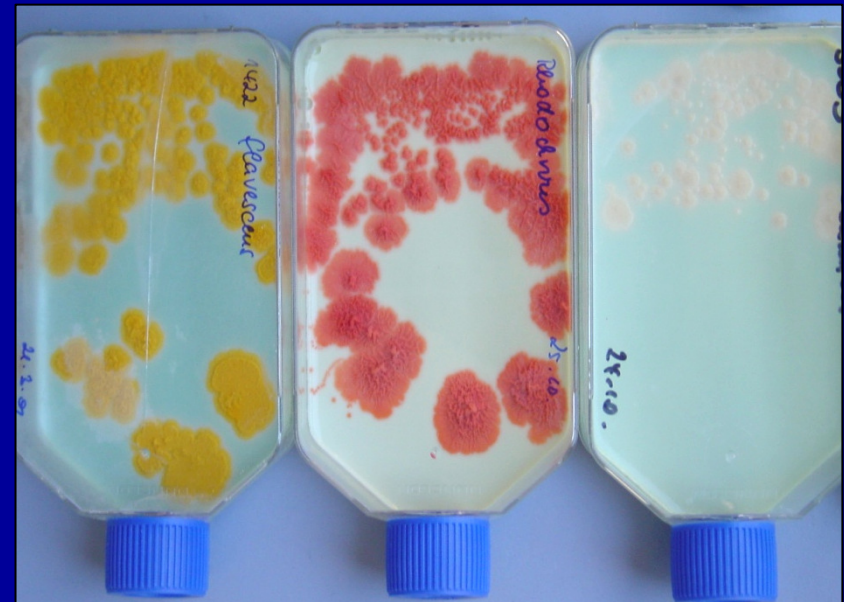
## Microarrays

Chiptechnology with fluorescence not routinely used for a clinical settings yet.



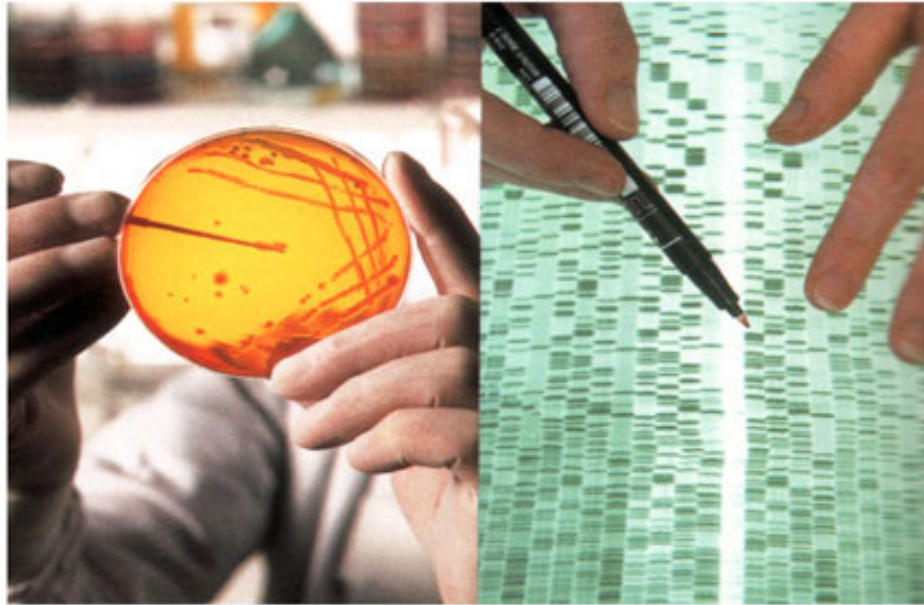
# Differentiation of Mycobacteria

- conventional  
(growth, colour, form, biochemistry)  
*2 - 4 weeks or longer*
- molecular  
(gene probes, PCR, sequencing 16S rRNA)  
*2 - 7 days*



<i>M. tuberculosis</i>	COGC <b>T</b> AAA <b>GC</b>	GCTTTCCACCA <b>C</b> AAGA	CATGC <b>A</b> T <b>CC</b>
<i>M. intracellulare</i>	COGC <b>A</b> AAA--	GCTTTCCACCA <b>A</b> AAGA	CATGC <b>G</b> T <b>CT</b>
<i>M. chelonae</i>	COGC <b>A</b> AAA--	GCTTT <b>G</b> CACCA <b>CTC</b> A <b>C</b>	CATGA <b>AGTC</b>
<i>M. smegmatis</i>	COGC <b>A</b> AAA--	GCTTTCCCC <b>TACCAGGC</b>	CATGC <b>GAC</b>
<i>M. xenopi</i>	COGC <b>TACCA</b> AAC	GCTTTCCACCA <b>CCCC</b> - A	CATGC <b>GCA</b>

# Hand in Hand



**Tradition and Hightech  
in TB Diagnostics**



# Diagnostic Tools: Assessment and Recommendations

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## Culture

- „Gold standard“  
most sensitive and specific
- Solid medium: limit of detection 100 bacteria, growth of TB after 4 weeks
- Broth: limit of detection 10 bacteria, growth of TB after ~2 weeks (e.g. MGIT<sup>®</sup>-System: **M**ycobacteria **G**rowth **I**nhibitor **T**ube)

## IS6110

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### For example:

Rapid diagnosis of tuberculous meningitis: a comparative evaluation of in-house PCR assays involving three mycobacterial DNA sequences, IS6110, MPB-64 and 65 kDa antigen

Rafi W et al.

J Neurol Sci 2007

Against a gold standard of culture, a sensitivity of 98% (NPV=99%) and a specificity of 100% (PPV=100%) was observed with the IS6110 PCR. Among the nested PCRs, a sensitivity of 91% (NPV=94%) and a specificity of 91% (PPV=85%) was observed with the MPB-64 assay.

## Real-Time-Sensitivity ?

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Comparison of real-time polymerase chain reaction using the Smart Cycler and the Gen-Probe amplified Mycobacterium tuberculosis direct test (MTD) for detection of M. tuberculosis complex in clinical specimens

*Pounder JI, Aldous WK, Woods GL  
Diagn Microbiol Infect Dis 2006*

Real-Time:	Sen 86.3%	Spez 100%	PPV 100%	NPV 94.5%
MTD:	Sen 98.0%	Spez 99.2%	PPV 98.0%	NPV 99.2%

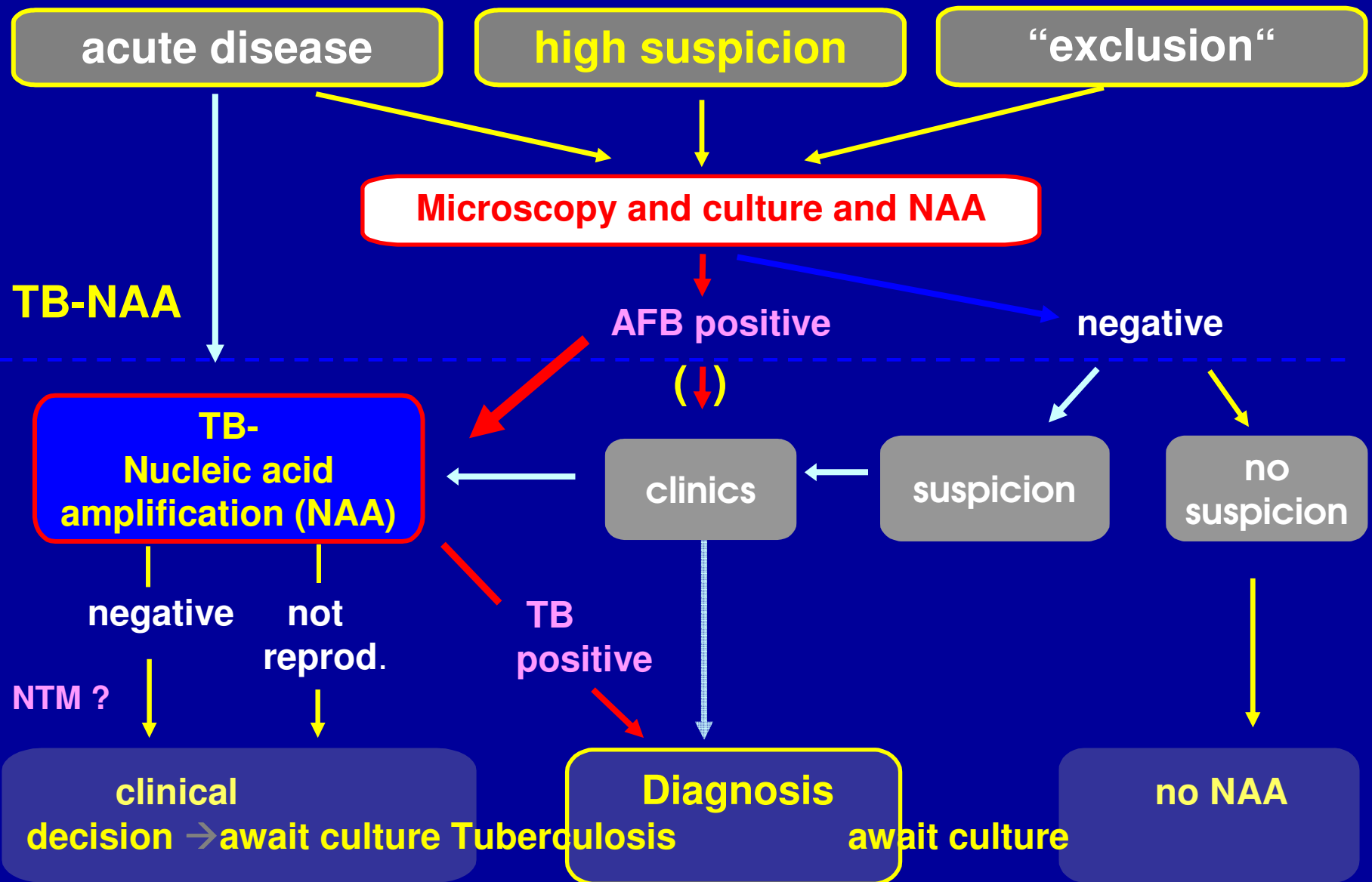
Comparison of an internally controlled, large-volume LightCycler assay for detection of Mycobacterium tuberculosis in clinical samples with the COBAS AMPLICOR assay

*Burggraf S, Reischl U, et al.  
J Clin Microbiol. 2005*

Sensitivity COBAS Amplicor = Real-Time

TaqMan Roche ?

# Workflow: Diagnosis of Tuberculosis using PCR





# Indication for PCR/ NAA Mycobacteriology

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**Sensitivity: culture (100%)  $\geq$  PCR (~ 90%)  $\gg$  smear (~50%)**

## Approved indication (routine laboratory)

- Respiratory tract secretions (tuberculosis)
  1. Microscopy positive (and AIDS)
  2. Microscopy negative and clinical suspicion of tuberculosis
- CSF
  3. Meningitis tuberculosa

## Special indications (reference laboratory)

- Extrapulmonary tuberculosis
- NTM