

# AFB Identification Texas Approach

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# DSHS-Austin TB Lab

## Customers & Samples Year 2009

- 175 public health clients
- 101 hospital/private clients
- Specimens for AFB smear & culture
  - 21,860; 60% public health, 40% hospital/private
    - 2,543 Mtb; 92% public health
    - 1,240 NTM; 76% public health
- Referred Isolates for ID/DST
  - 1,888; 45% public health, 55% hospital/private
    - 1,440 NTM; 44% public health
    - 351 Mtb; 56% public health
  - 1,385 isolates for Mtb genotype

# TX ID Strategy

- Direct ID by HPLC & MTD
- HPLC as primary DX for Mtb from culture
  - Occasional probe/biochemical
  - NTM reported often as groups
  - Refer on request NTM for further ID/DST

# TX HPLC Method

- Fluorescence-HPLC based on the CDC UV-HPLC
- Direct broth saponification
  - At day=0
  - No centrifugation >> faster >> safer
  - Autoclaved >> biosafety
- In vial derivatization
  - Reduced cost and labor
- MAC external stnd to replace internal stnds
  - Calibration control for instrument and peak names
  - Positive control batch
  - Carryover control

# TX HPLC Practice

- Performed daily
- All smr+ diag. specs. & AFB culture +
- Immediately on ref cults w/o standardized growth conditions
- Substitutes for culture smear & eliminate “AFB found on culture, ID pending” reports
  - Removes one reporting layer
  - Replace non-specific report with a specific one

# 336 Instrument + MGIT 960 Cultures

HPLC result	AFB smear result	
	Negative	Positive
M. species	8*	224
Inconclusive	96	8**
Total	104	232

\* final culture result: 4 MTB, 4 NTM

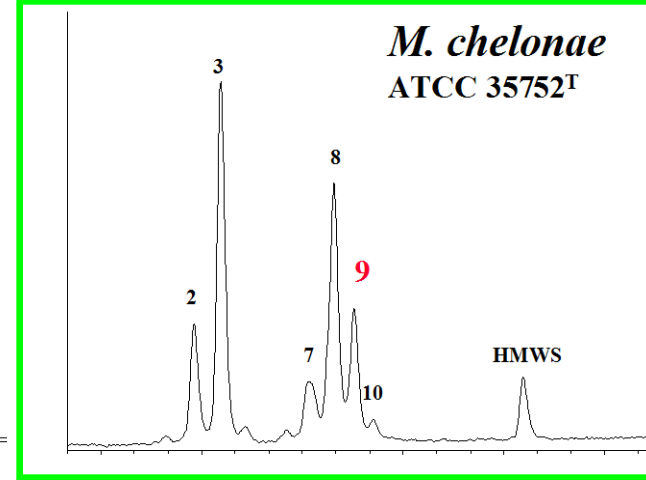
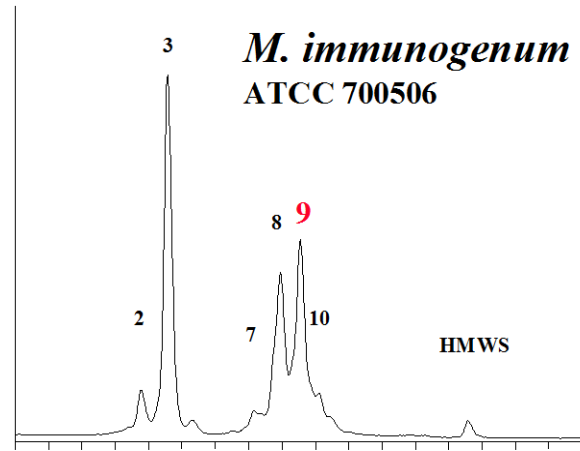
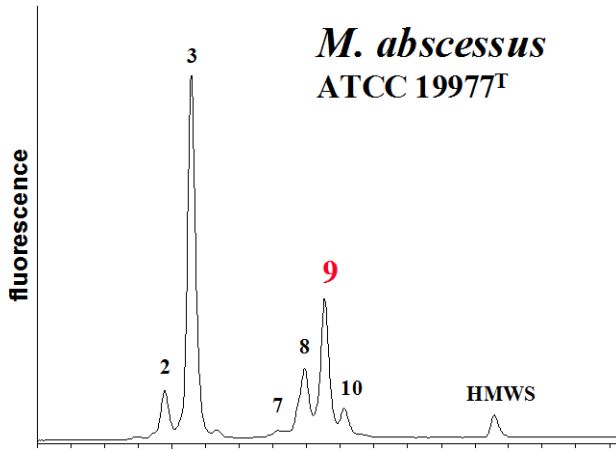
\*\*final culture result: 4 AFB neg, 3 contam, 1 NTM

# HPLC & Complexes/Groups

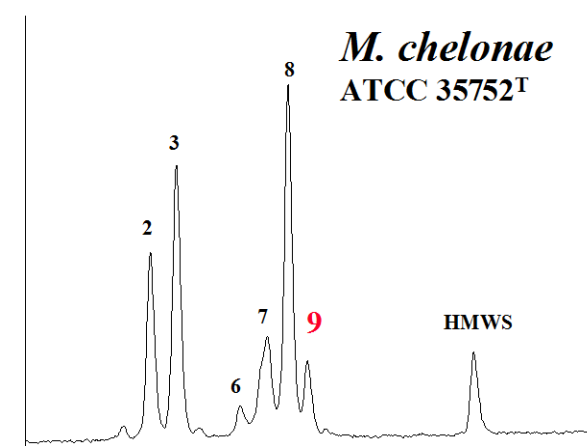
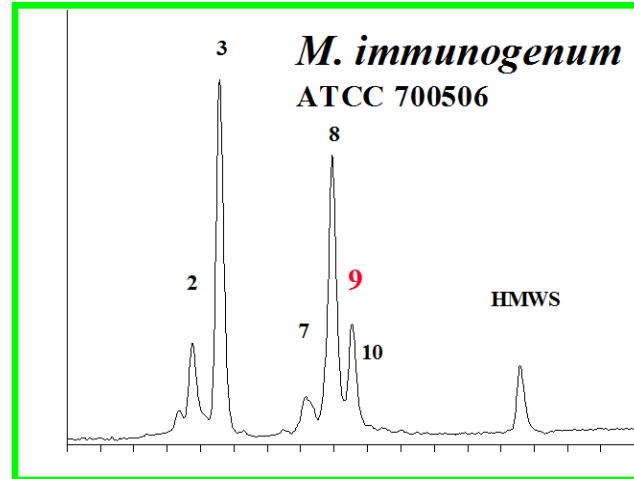
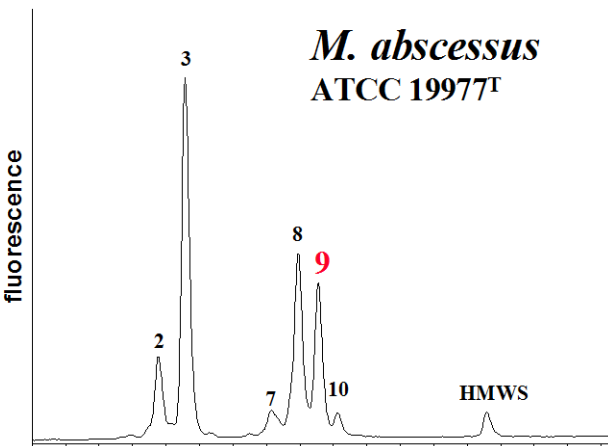
- More than 140 NTM
  - >40 associated with disease
- *M. fortuitum* group
  - Expanded to *M. fortuitum-smegmatis* group
- *M. simiae*
  - *M. simiae-lentiflavum-triplex* complex
- Upon request, refer overnight for molecular ID or NTM DST
  - pay shipping costs

# Incubation Temp Effect

## 35°C LJ



## 30°C LJ



# Reported based on HPLC alone (including from broth cultures)

- *M. tuberculosis* complex
- *M. avium* complex
- *M. fortuitum*-*smegmatis* group
- *M. abscessus*-*chelonae* group
- *M. gordonae*
- *M. kansasii*
- *M. simiae* complex
- *M. nonchromogenicum*
- *M. flavescens*
- *M. xenopi*
- *M. marinum*
- 12 species, cx, or groups;  
89% of all ID reports
- 84% of Mtb+ initial Dx specs  
reported  $\leq 21$  days

# Reported based on HPLC plus

## Morphology:

- *M. mucogenicum*
  - *M. terrae* complex
  - *M. kubicae*
  - *M. haemophilum*
  - *M. asiaticum*
- 
- 6% of all ID reports

## Select biochems:

- *M. abscessus*
- *M. immunogenum*
- *M. chelonae*
- *M. szulgai*



# What's left?

- Nontuberculous Mycobacteria
  - Pigmented rapid grower
  - Non-pigmented rapid grower
  - Pigmented slow grower
  - Non-pigmented slow grower
  
- 4% of all ID reports

# NTM Referrals for ID/DST

- In 2009
  - 66 isolates referred to U. of TX Health Tyler
    - 45% for rapid growers
    - 27% for MAC
    - 27% for other NTM
  - Isolates are sent day after request at no charge

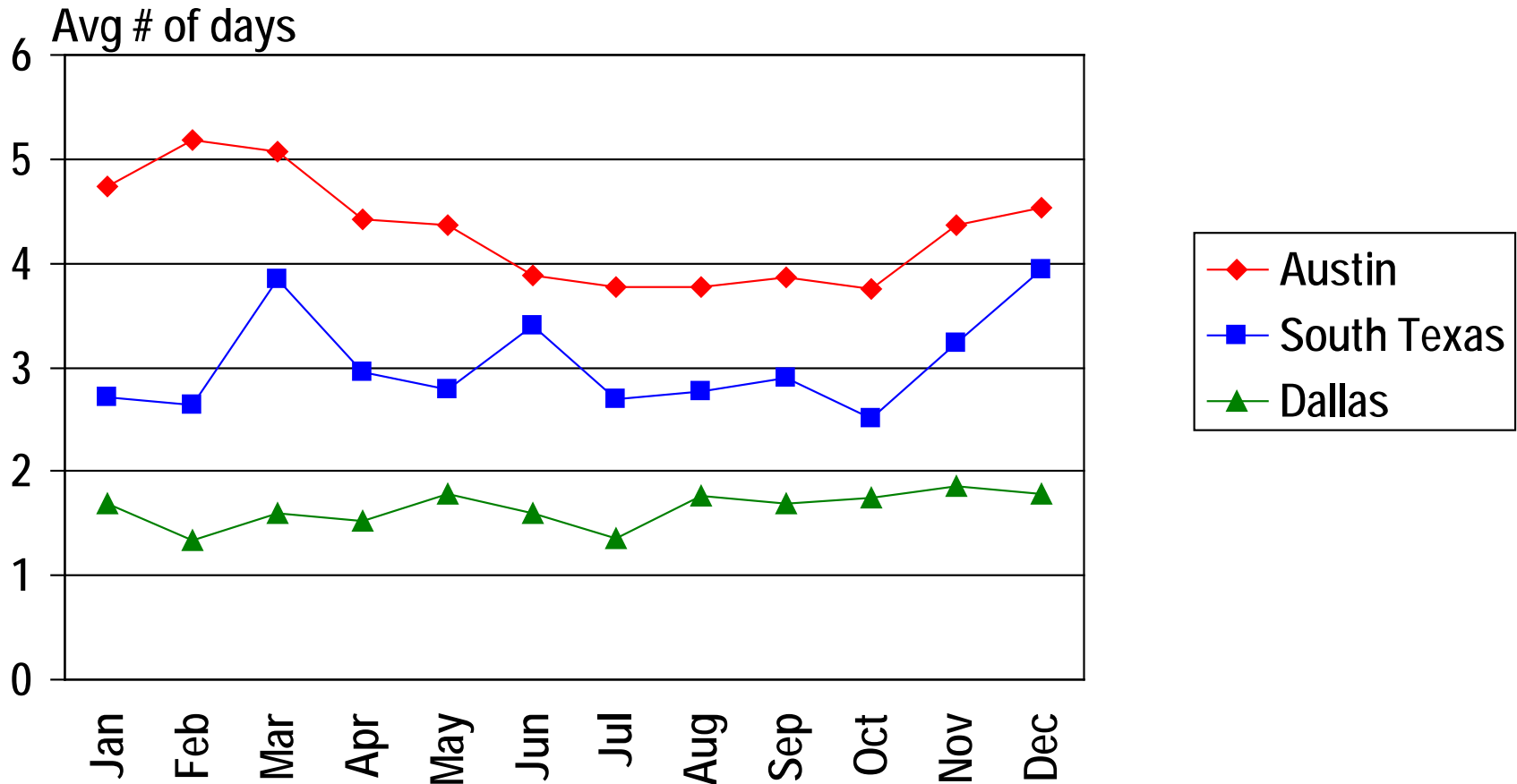
# Direct Identification



TX PH TB Lab	# of specimens for AFB Smear & Culture 2009
Austin	21,860
Houston	7,121
Tyler	3,775
Harlingen	3,044
San Antonio	2,823
Dallas	2,215
El Paso	609

# Specimen Collection to Lab Receipt

## Jan – Dec 2009



# Direct Identification

- MTD Package insert
  - “Specimens must be collected in sterile plastic containers, and stored at 2° to 8°C until transported **or** processed.”
  - “Final specimen concentrations of NaOH other than 1 to 1.5% should not be used for processing specimens to be tested.”
- CLIA Region 6
  - We performed & presented NaOH validation studies; Region 6 never acknowledged
- Direct HPLC on smear positive
  - In 2009, 414 cult+ tb patients; 129 (31%) id’d by DLC

# HPLC Summary

- *Advantages:*

- Rapid ID
- Simple sample prep
- Low materials cost, <\$1/test
- Accurate ID of MTBC
- Reliable ID of some NTM

- *Disadvantages:*

- HPLC equip costly
- Redundant system needed?
- Must be complemented by NAA
- Technical expertise required



# Next Steps

- Courier for improved specimen delivery
- Restore NAA
  - 2010 CAP accreditation
  - LDT?
- Customer Needs Survey (NTM ID!)
- Molecular DST

The End!

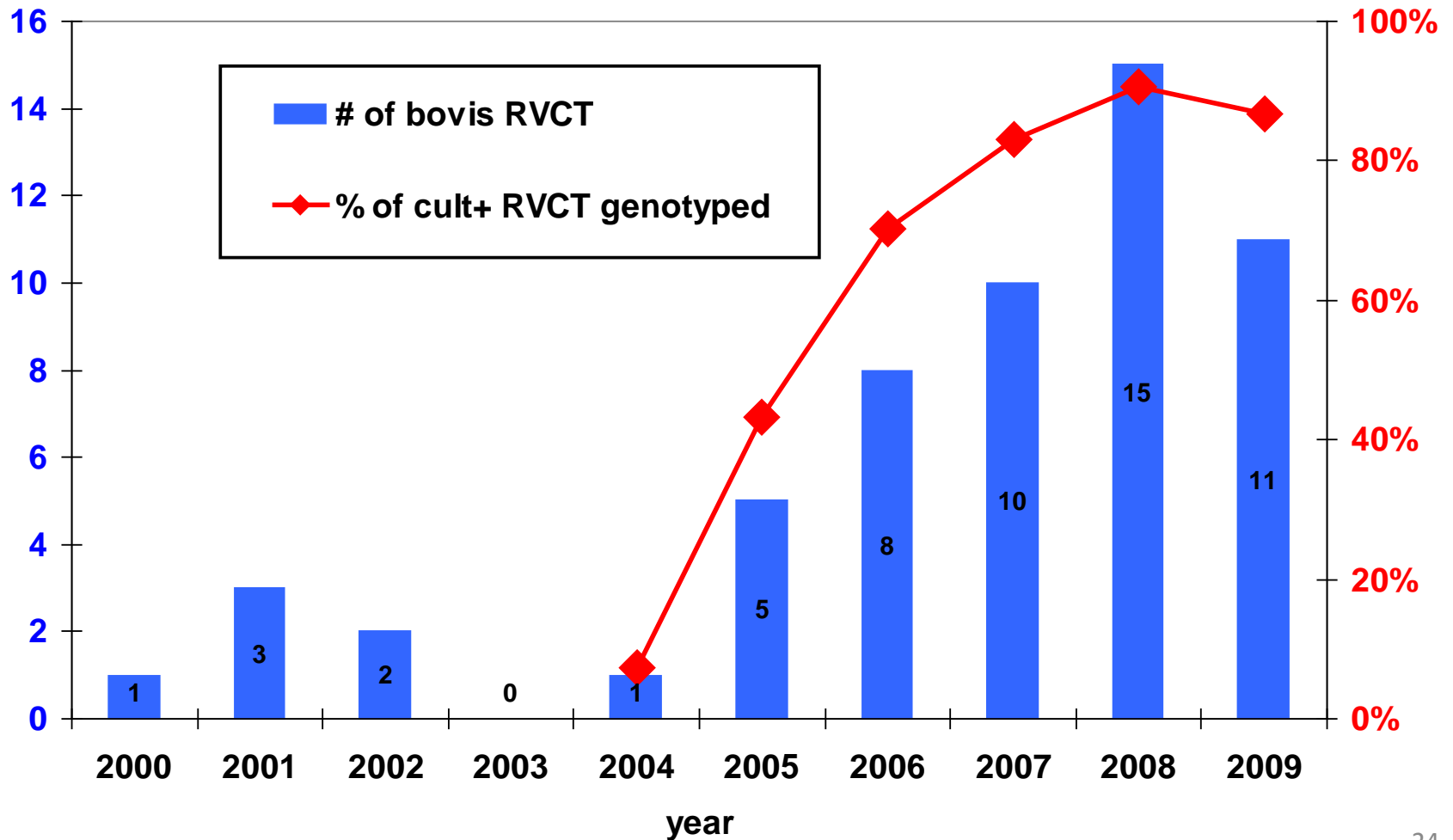
Thank you



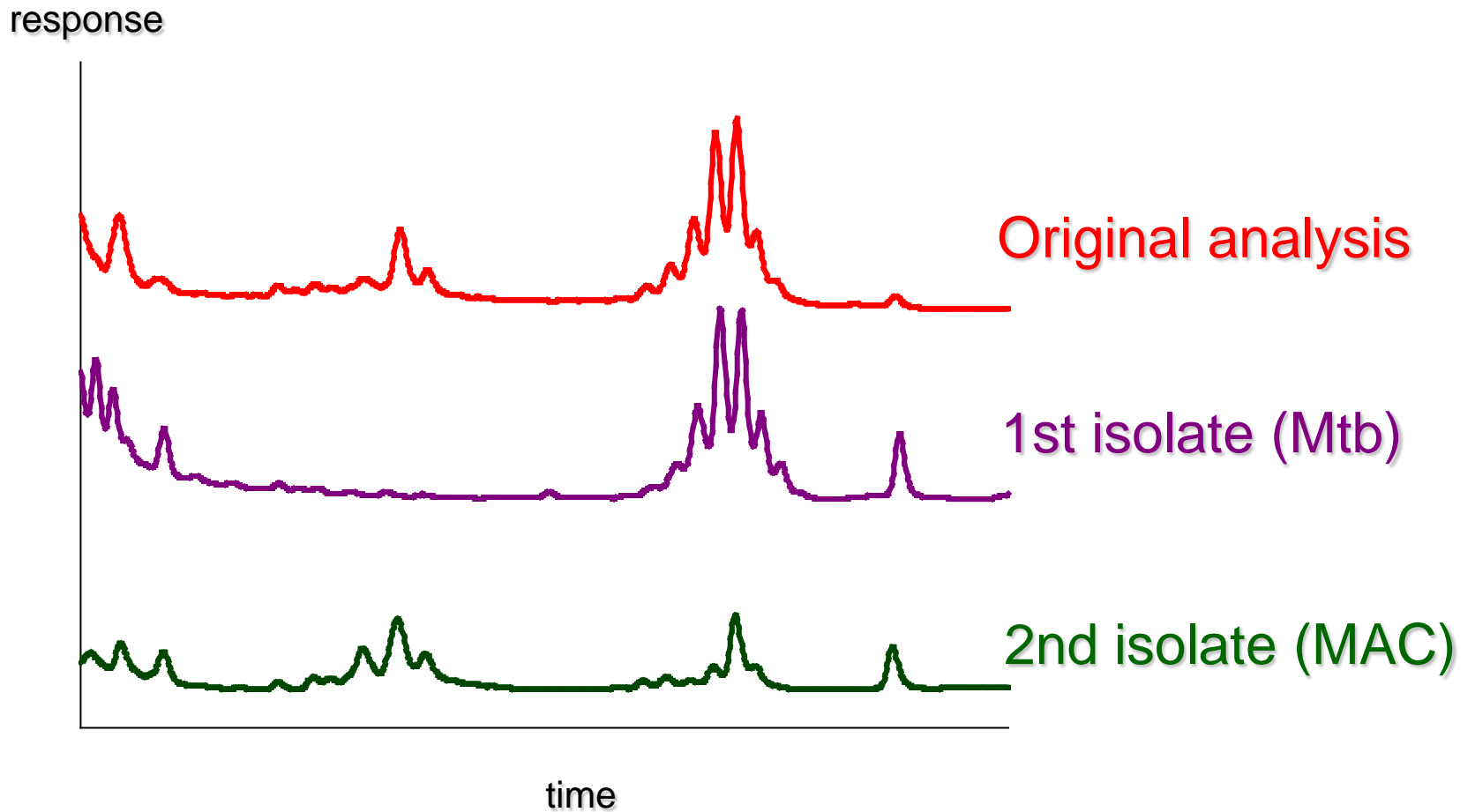
# Backup

	TB Cases		
Yr 2008	US	Texas	TX % of US
all TB	12904	1501	12%
cult+ TB	10030	1183	12%

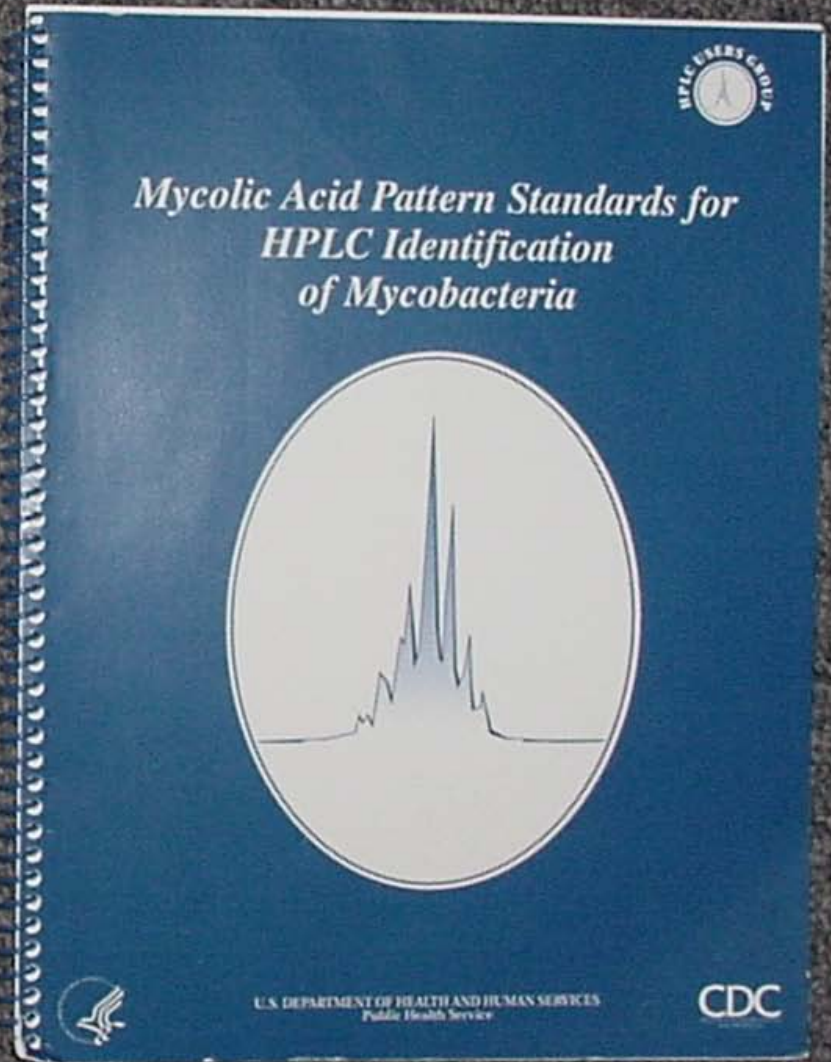
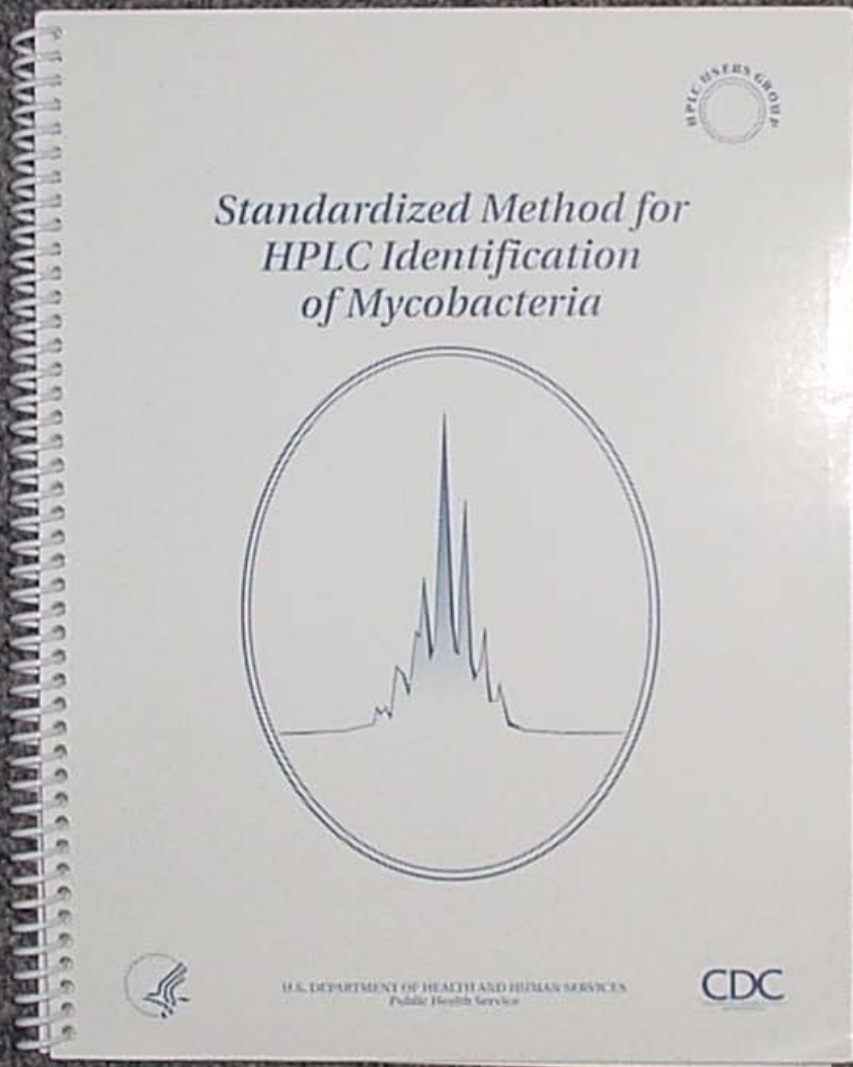
# M. bovis & Genotyping in Texas



# Mixed Culture



# CDC/HPLC Users Group



<http://www.mycobactoscana.it/patterns.htm>



## Mycobacteria Reference Center - Tuscany

Region of Tuscany Resolution 411, March 28, 2000

[Home Page](#)

[Diagnostic activity](#)

[Epidemiology](#)

[HPLC](#)

[Info](#)

[Links](#)

[Scientific activity](#)

 [in italiano](#)

SEARCH

### SCIENTIFIC ACTIVITY

- Next meetings
- International collaborations
- New mycobacterial species the center collaborated to describe
- Publications

### DIAGNOSTIC ACTIVITY

- The tests performed in the center



★ add to Favorites

### EPIDEMIOLOGY

Epidemiological information concerning the cases of microbiologically diagnosed tuberculosis since 2001 in Tuscany

Among indicators investigated:

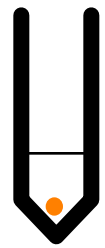
- the distribution of cases among different age groups
- the proportion of cases among Italians and foreigners
- the drug resistance of the strains
- the MDR strains
- the worrying Beijing strains

# HPLC Sample Preparation

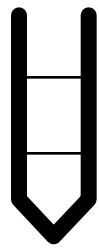
**SAPONIFY**

**EXTRACT**

**DERIVATIZE**



**AUTOCLAVE**



**XFER CHCl<sub>3</sub>**



**HPLC**

**BACTERIA**

**+**

**KOH**

**HCl**

**+**

**CHLOROFORM**

**DERIVATIZING**

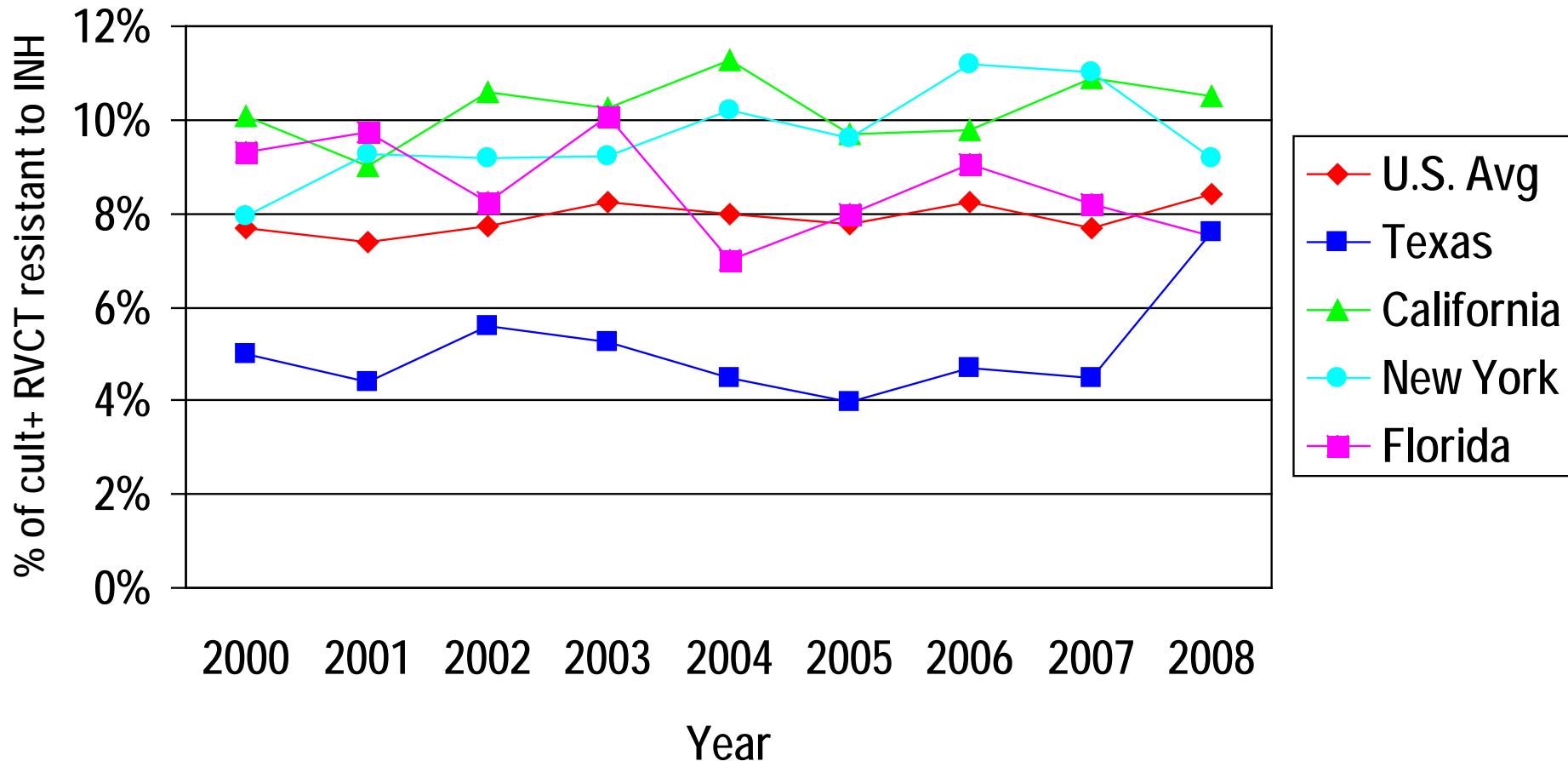
**REAGENT**

# Standardized Method for HPLC Identification of Mycobacteria

- NCCLS format
- Consensus guidelines for UV analysis
- Standardized sample prep
- HPLC with generic system
- QC
- Aligned reference patterns of 19 species/groups

# Culture + RVCT Resistant to INH

Texas Drug Resistance Testing & Reporting Policy Modified 1-1-08



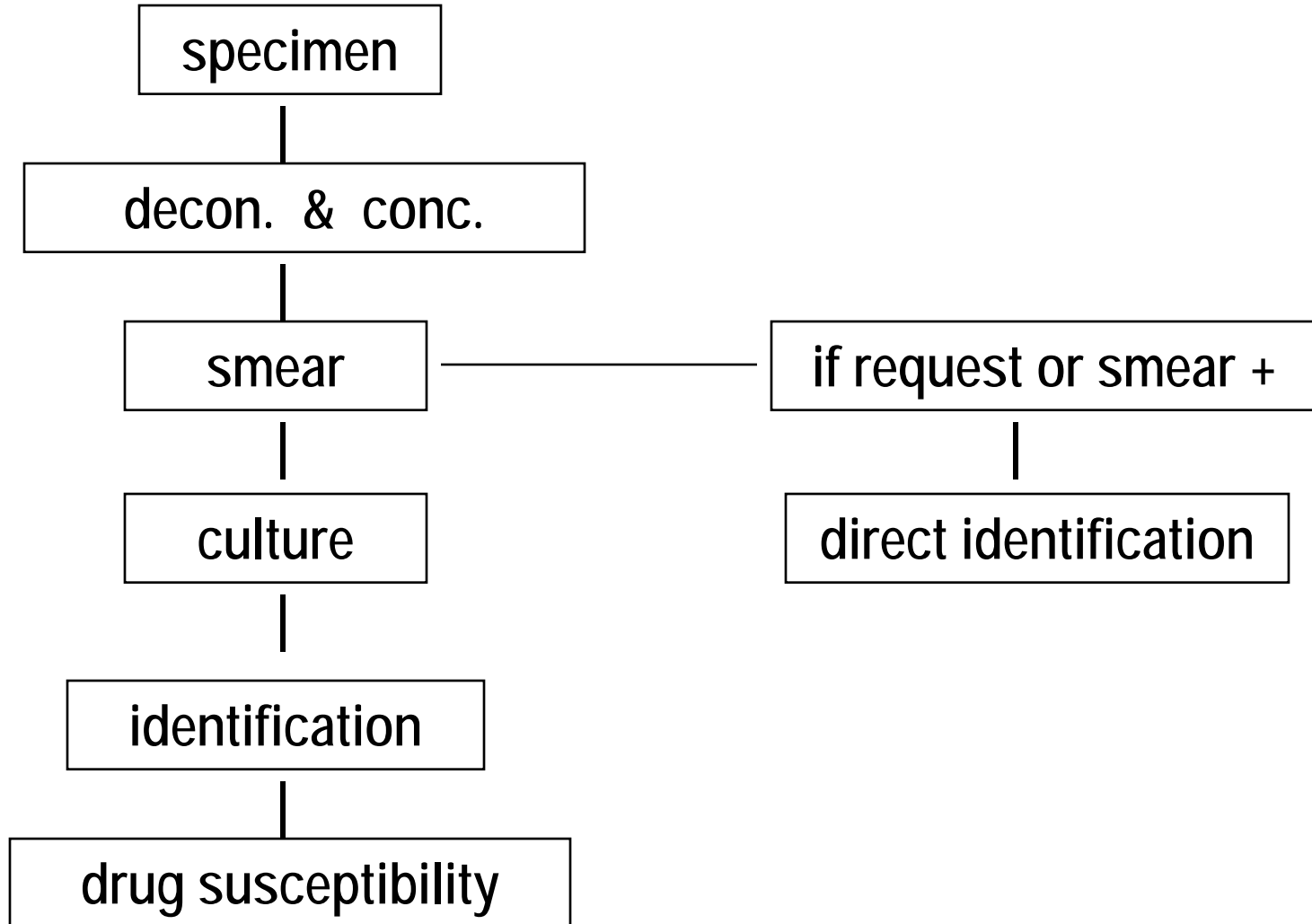
# DSHS Austin Patient Specimen Criteria for Reflex Direct Identification

- Smear Positive
- No culture-positive TB within 1 year
- No multi-drug treatment 7 day-1 year
- No smear positive NTM or  $\geq 3$  smear negative with same NTM within 1 year
- For NAAT
  - Respiratory source only
  - Smear negatives on request

# AccuProbes for Mycobacterial species

- ACCUPROBE® for Mycobacterium species
  - *M. tuberculosis* complex
    - *M. avium* complex
    - *M. kansasii*
    - *M. goodii*
    - *M. avium*
    - *M. intracellulare*
- All are separate probes
  - Selected for testing based on growth rate of colonies, cord formation, pigmentation

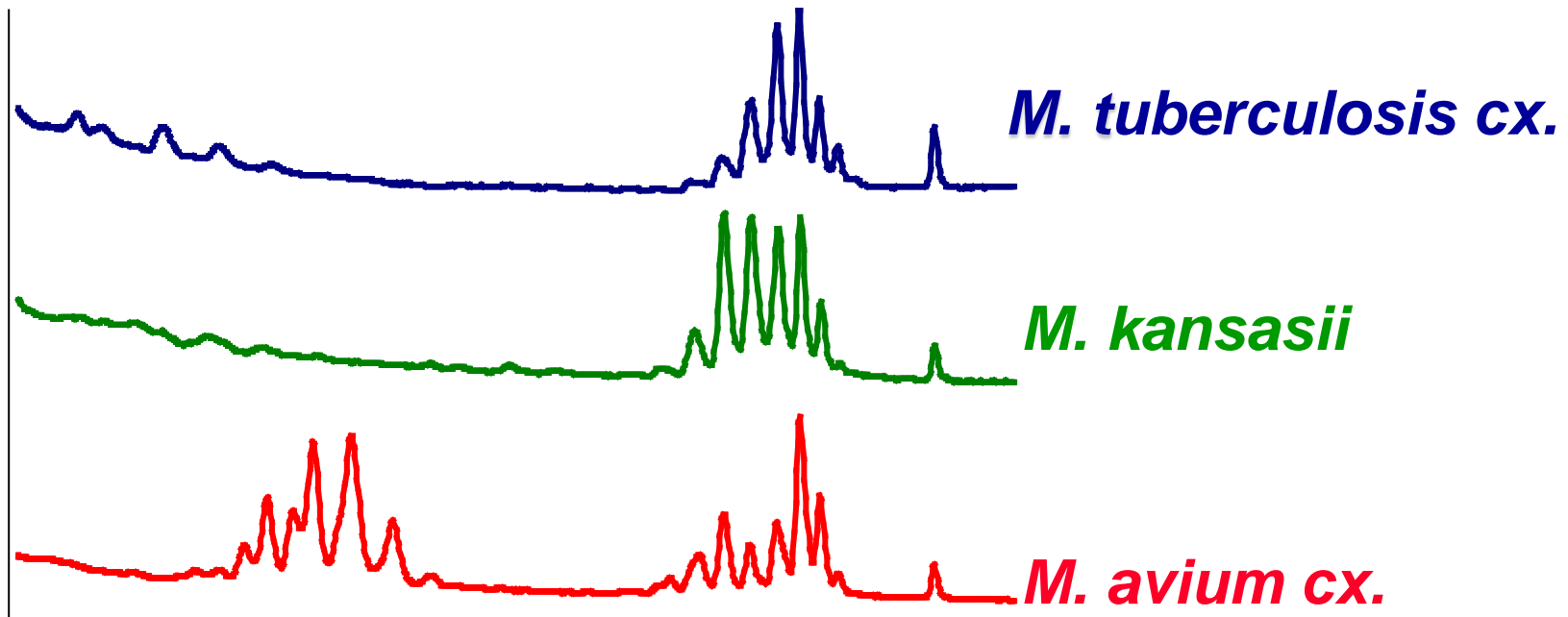
# Identification



# Fluorescence-HPLC Patterns

Directly from Smear+ Clinical Specimens

response



retention time

# Mixed Cultures

- If Mtb found, NTM not routinely worked up
  - Expert docs, TB Prog, HIV
  - May be worked up if dst delay