Laboratory services for the diagnosis and management of TB in HIV co-infected patients

Max Salfinger, Wadsworth Center

Fogarty Symposium on TB/HIV

Cali, Colombia - April 6, 2006

John E. Fogarty, Bricklayer & Rhode Island Congressman

1913 - 1967

‘Time and again, it has been demonstrated that the goal of better health has the capacity to demolish geographic and political boundaries and to enter the hearts and minds of men, women and children in the four corners of earth. It is an issue which serves as a forceful reminder of the oneness, the essential brotherhood of man.’

Laboratory's charge:

To provide the clinician/TB controller with accurate results in a timely fashion!

Patient sees a doctor

Adherence/Cure

3 Time to negativity?

2 RIF resistance?

1 Tuberculosis?

Chest x-ray

History / physical exam

Inhalation of 1-5 mm Ø droplets

No infection

10-30% infection

90% LTBI

5-10% acute TB or TB within 2 years

10% TB during lifetime
10% TB within 1 year if HIV +

HIV -

85% pulmonary TB
15% extrapulmonary TB

HIV +

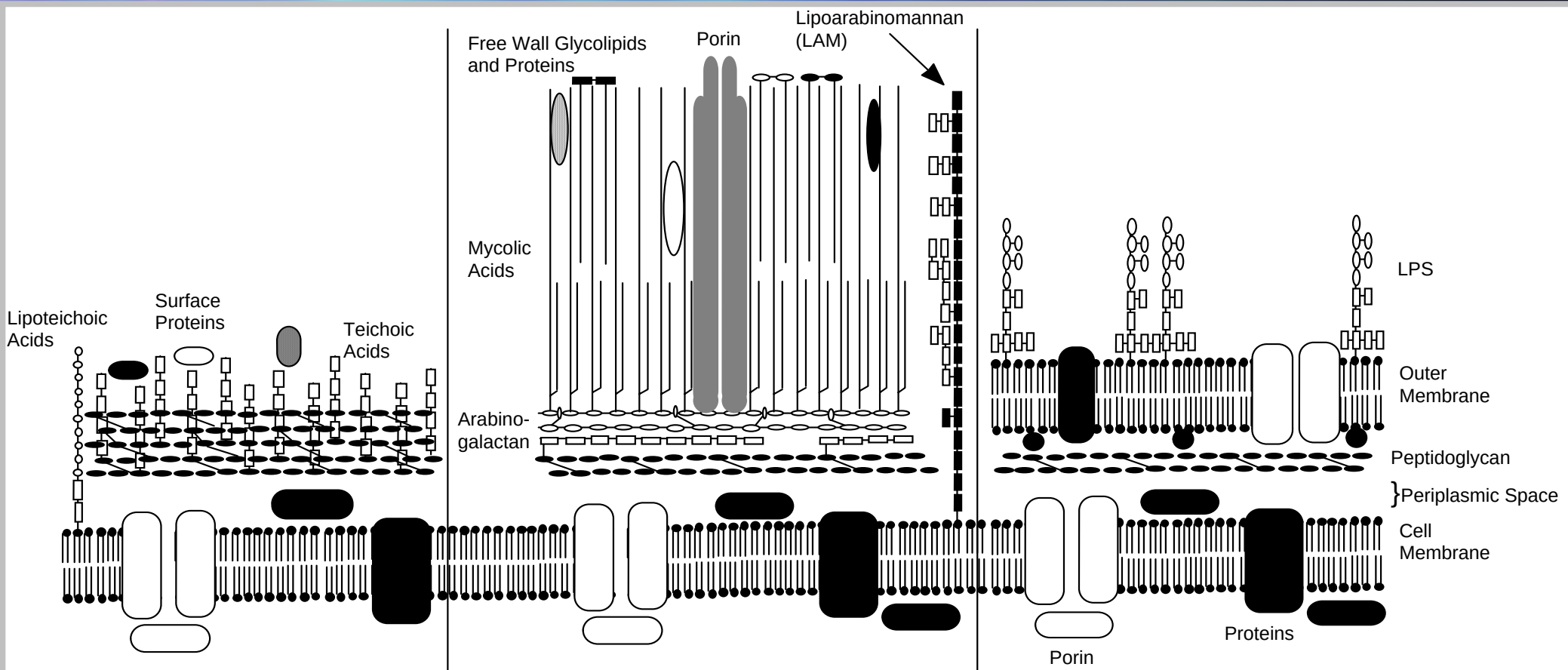
33% pulmonary TB
33% extrapulmonary TB
33% both

Collection & transport

- Quality testing requires quality specimen
- 5 to 10 ml sputum
- **If HIV+:**

  blood cultures

Cell wall and generation time differences of Gram-Positives, Mycobacterium and Gram-Negatives



Gram-Positive	Mycobacterium	Gram-Negative
20 Min	**20 Hours**	**20 Min**

Parsons LM et al 1997 Infect Dis Clinics NA 11:905-928

TB-HIV

Adults with a Fever:

	HIV	TB
Malawi	79%	11%
Thailand	71%	9%

McDonald et al Lancet; 354(9185):1159 163(1999)

Adults with a Fever:

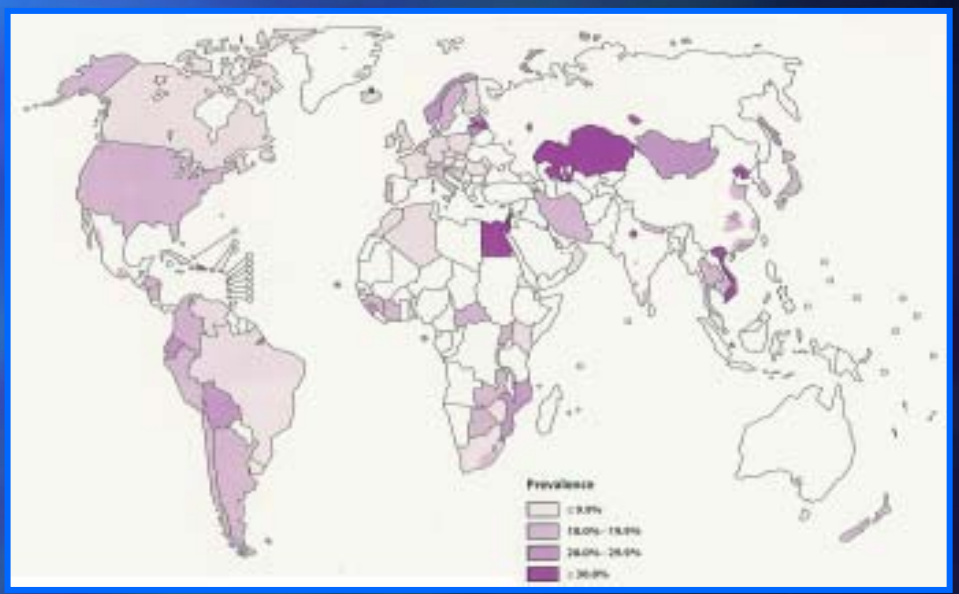
	HIV	TB	MDR
Malawi	79%	11%	0%
Thailand	71%	9%	25%

McDonald et al Lancet; 354(9185):1159 163(1999)

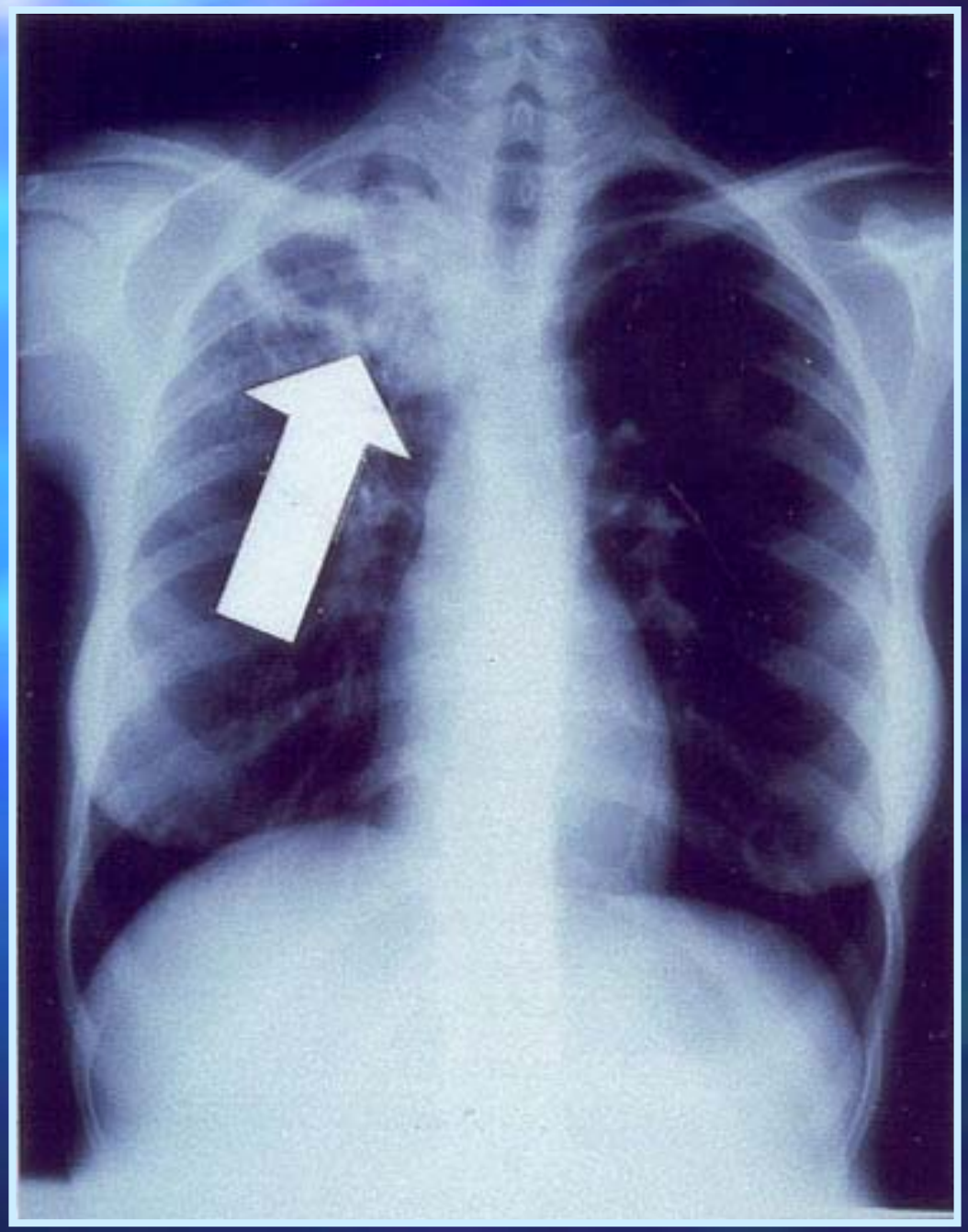
Resistant and MDR TB

- WHO estimates that **50 million** are infected with resistant tuberculosis

- **Poorly functioning TB programs** create drug resistant TB:
  - The wrong drugs or combination of drugs are prescribed
  - The adequate drugs are not taken consistently
  - The adequate drugs are not taken for the entire 6 months of treatment



Faster Turnaround Times !



Tuberculosis

yes

or

no ?

Identification

Growth detection

Microscopy

Nucleic acid amplification

Microscopy

- Ziehl-Neelsen & fluorochrome
- 5,000 to 10,000 bacilli per ml for a positive smear
- Results within 24 h
- In HIV+ less sensitive

Quality control of smear microscopy for AFB: the case for blinded re-reading

Lan et al, Int J Tuberc Lung Dis 1999, 3:55-61

Protocol:

- 3 Provincial labs, ea. 750 slides
- 2 Techs ea. 375 slides
- 3 Study arms ea. 125 slides:
  unblinded, unblinded-misclassified, blinded

Results:

- **Unblinded:**

  2.9 % false-neg; 0% false-pos

- **Unblinded/mislabeled:**

- **Blinded:**

  18.7% false-neg; 0% false-pos

Results:

- Unblinded:

  2.9 % false-neg; 0% false-pos

- Unblinded/mislabeled (61 weakly pos as neg*):

  11.3% (*39%) false-neg; 0.2% false-pos

- Blinded:

  18.7% false-neg; 0% false-pos

NAA

Nucleic acid amplification

- FDA approved:

  Smear-pos (Dec 1995)
  Smear-neg* (Sep 1999)

- MMWR July 7, 2000 [R]
- AFB-pos / NAA-neg
- AFB-neg* / NAA-pos

Potential public health implications:

38 year old male from Puerto Rico seeks care in US.

Fast Track Specimen 202413:

10-05/10:55 AM Lab receives phone call from ICN:

AIDS pat flew from Puerto Rico to Newark on 9-30, directly admitted to Long Beach MC, intubated 10-02

10-05/11:00 AM Specimens arrives at Wadsworth Center

10-05/1:00 PM Concentrated smear: moderate AFB

10-05/2:53 PM Nucleic acid amplification assay: negative

10-05/5:51 PM Repeat NAA assay: negative, no inhibition

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10-10/11:00 AM BACTEC vial grew *M. avium* complex

MTD Analysis:

- July 1998 - June 2002
- 1561 specimens
- Sens 98.2%; Spec 97.2%
- PPV 97.2%; NPV 98.2%
- 21 MTD pos, culture neg

Wadsworth Center Data (ASM 2003 abstract)

MTD analysis after 21 patient charts review:

- July 1998 - June 2002
- 1561 specimens
- Spec 100 %
- PPV 100 %
- 0 true false positive MTD

Culture

Gold Standard: Solid <u>and</u> liquid medium

Processing sputum

- Procedures kill all but 10 to 20% of the mycobacteria
- Contamination: 2 to 5% of sputum specimens on Lowenstein-Jensen medium (LJ)

Growth detection

- 78% culture-pos TB cases in 2004
- Solid and liquid medium (a must for HIV+)
- Commercial broth system
- Smear-pos / culture-neg

Bactec 460TB and MGIT 960 growth detection and DST systems

- **Bactec 460 TB system**
  - shorter TAT
  - semi-automated
  - radioactive
  - requires needles
  - special gas mix

- **MGIT 960 system**
  - fully automated, walk-away
  - non-radiometric
  - no need for needles (inoculation and testing)
  - no need for manual loading of vials
  - no need to establish reading schedules

Identification

- **116** species in genus *Mycobacterium* as of Jan '06
- ***M. tuberculosis* complex**

(*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, etc)

NAA, AccuProbe, and 16S sequencing detect all members of *M. tuberculosis* complex

- *M. tuberculosis*
- *M. bovis*
- *M. bovis* BCG
- *M. africanum*
- *M. caprae*
- *M. microti*
- *M. canettii*
- *M. pinnipedii*

Drug susceptibility testing or detection of drug resistance

Drug susceptibility testing

- On all initial M.tb isolates (2004: 93.9%, US)

SIRE + **PZA**

- Faster with radiometric (fastest with *rpoB* analysis)
- Confirmation of drug resistance

Testing concentrations:

Drug	460TB	MGIT	7H10	7H11
INH	0.1/0.4	0.1/0.4	0.2/1.0	0.2/1.0
RIF	2.0	1.0	1.0	1.0
EMB	2.5/7.5	5.0	5.0/10	7.5
SM	2/6	1/4	2/10	2/10
PZA	100	100	N/A	N/A

RIF resistance

yes

or

no ?

Clinical course

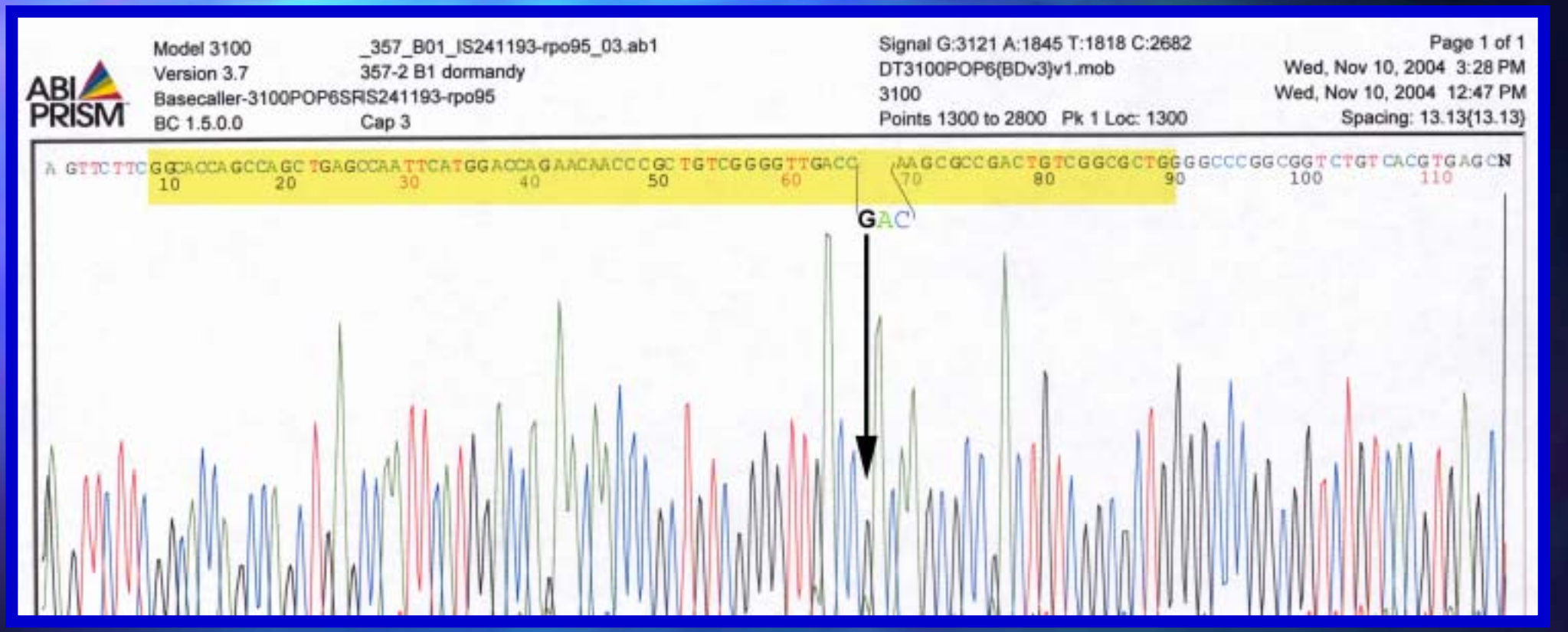
Egg based AST

Agar based AST

Radiometric / Non-r.

rpoB analysis

rpoB analysis



Codon 526 (CAC) encodes histidine in susceptible strain replaced with (GAC) aspartate in resistant strain.

Further DST solution

JOURNAL OF CLINICAL MICROBIOLOGY, July 2003, p. 2822–2826 Vol. 41, No.
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.7.2822–2826.2003

Use of Molecular Methods To Identify the *Mycobacterium tuberculosis* Complex (MTBC) and Other Mycobacterial Species and To Detect Rifampin Resistance in MTBC Isolates following Growth Detection with the BACTEC MGIT 960 System

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Received 29 January 2003/Returned for modification 10 March 2003/Accepted 14 April 2003

- Use of molecular assays in combination with broth-based systems

Molecular testing:

Drug	Gene	% mutations
RIF	*rpoB*	>96%
PZA	*pncA*	97%
INH	*katG*	40-60%
INH-ETH	*inhA*	15-43%
INH	*ahpC*	10%
INH	*kasA*	unknown

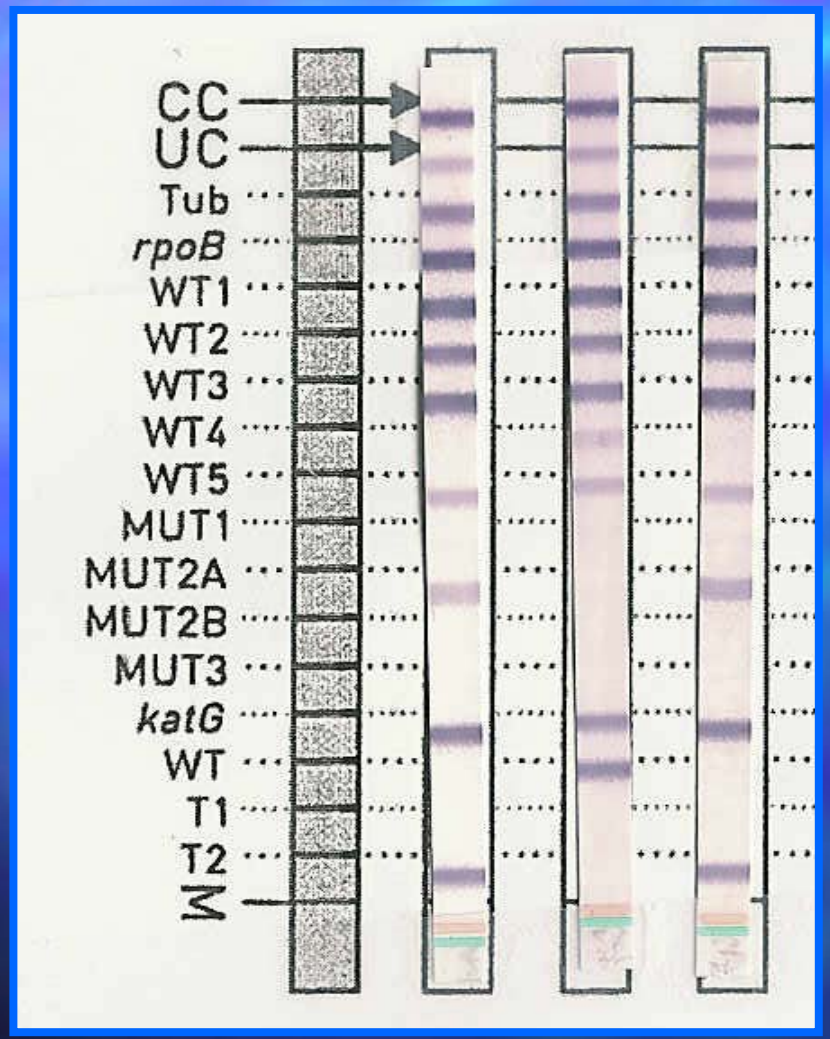
Drug-Resistant TB -

A Survival Guide For Clinicians

Francis J. Curry National Tuberculosis Center, San Francisco, 263 p. (2005)

www.nationaltbcenter.edu

Latest development



Haine Lifescience

Haine Lifescience GenoType MTB-DR*

- Excellent performance on frozen sputum sediments (< 6 years)
- Overall TAT: **2 days**
- Sensitivity for MTBC is similar to MTD or Amplicor
- INH_{high} resistant: 84% *katG* pos
- RIF resistant: 96% *rpoB* pos

*Wadsworth Center: Unpublished data

Patient sees a doctor

Adherence/Cure

3 Time to negativity?

2 RIF resistance?

1 Tuberculosis?

Chest x-ray

History / physical exam

Follow up specimens I

- Follow up specimens until 2 consecutive specimens are culture negative:
- AFB smear negative: at least once a mo
- AFB smear positive: **bi-weekly**

2 sputum specimens per event (NYS)

Follow up specimens II

- Follow up specimens until 2 consecutive specimens are culture negative...
- Initial cavitation & mo-2 culture pos: extend INH/Rif from 4 to 7 months
- Repeat susceptibility testing after 3 mo
- Pos culture @ mo-4: Treatment failure

TB fingerprinting

What have been the most useful aspects of universal DNA fingerprinting of M.tb?

- Detecting false positive cultures
- Uncovering previously unrecognized cases of transmission
- Assessing efficacy of TB Control programs

TB treatment:

80s: DOT (Union)

‘91: DOTS (WHO)

DOTS

I) Sputum smear positive

II) Taking pills under supervision

III) Complete treatment

IV) Right length of treatment

V) Commitment of government

WHO / IUATLD:

Initial: Sputum microscopy

If **positive**, then **treat**

@ Month 3: Sputum microscopy

If **positive**, then **culture** and **AST**

TB treatment:

80s: DOT (IUATLD)

'91: DOTS (WHO)

'99: DOTS-Plus*

*Farmer & Kim BMJ 317:671 674(1998)

DOTS-Plus

I) Second-line drugs availability

II) Drug resistance results in real time

WHO / IUATLD:

Initial: Sputum m scop

If **positive**, eat

@ Month 3: Sput copy

If po then ure and **AST**

THE GLOBAL PLAN

TO STOP TB

2006-2015

Actions for Life

TOWARDS A WORLD FREE OF TUBERCULOSIS

Stop TB Partnership

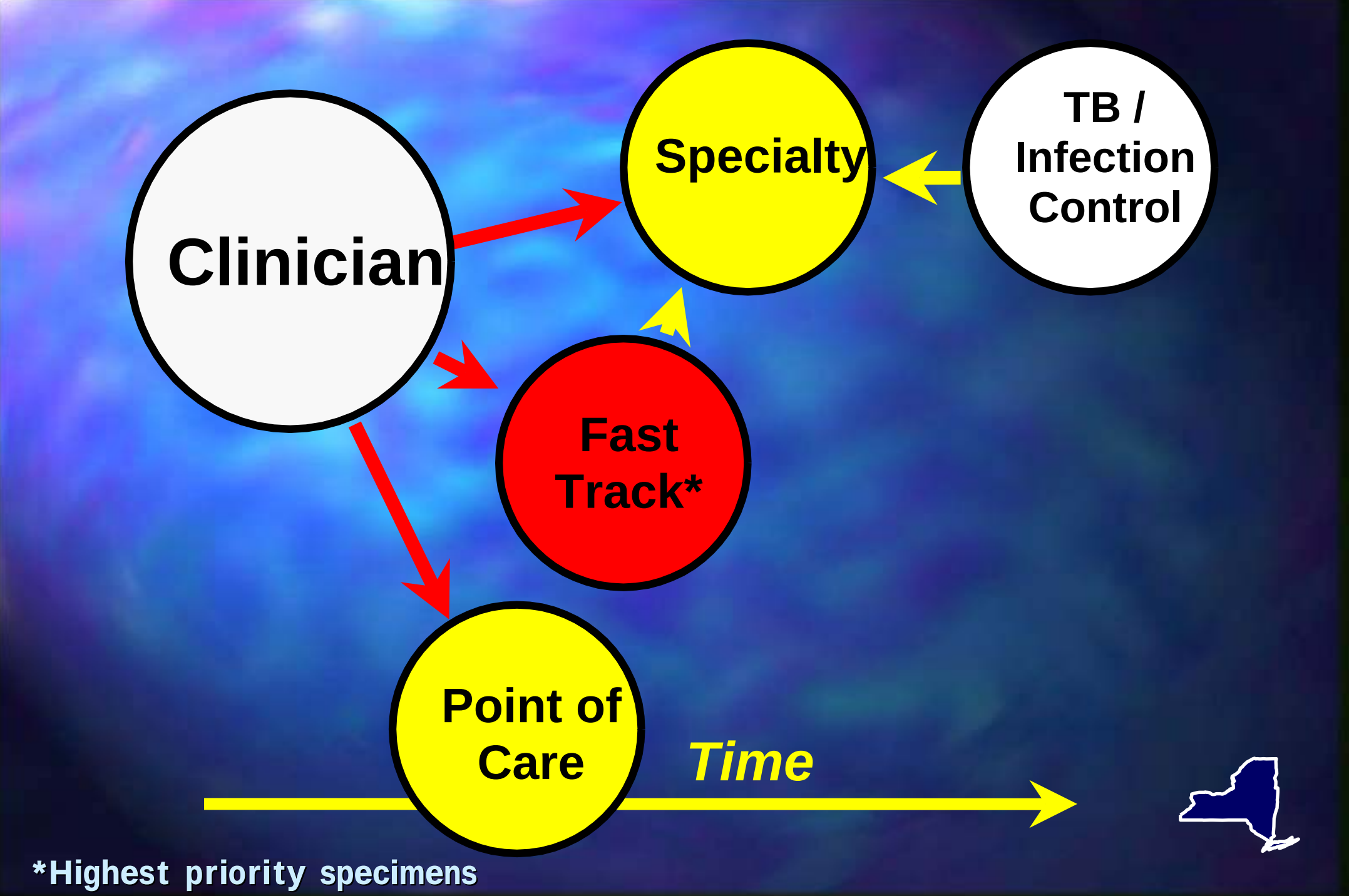
Actions for life towards a world free of tuberculosis:

1) Will expand equitable access for <u>all</u> to quality TB diagnosis and treatment

Is like conquering the Himalayan mountains


Genotyping
Resistance Detection
Growth & Identification
MICROSCOPY
• direct or concentrated
• Ziehl-Neelsen or fluorochrome stain
• solid or liquid based
• universal or selective
• agents to be tested
• solid or liquid or molecular based
• universal or selective
• real time retrospective
• IS6110 or PCR-based
• universal or selective
• real-time or retrospective
Requires additional resources!

In closing, a paradigm shift is warranted


Clinician
Specialty
TB / Infection Control
Fast Track*
Point of Care
Time
*Highest priority specimens

Let's be radical:

Move from AFB microscopy to molecular detection of MDR TB in sputum samples without first growing the tubercle bacilli!

- Fighting TB
- Fighting poverty
- Standing up for

PEACE On Earth!

Obrigadoh!

Tesekkür ederim!

Gracias!

Spacibo!

Merci!

Grazie!

Danke!

Thank you!