



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!

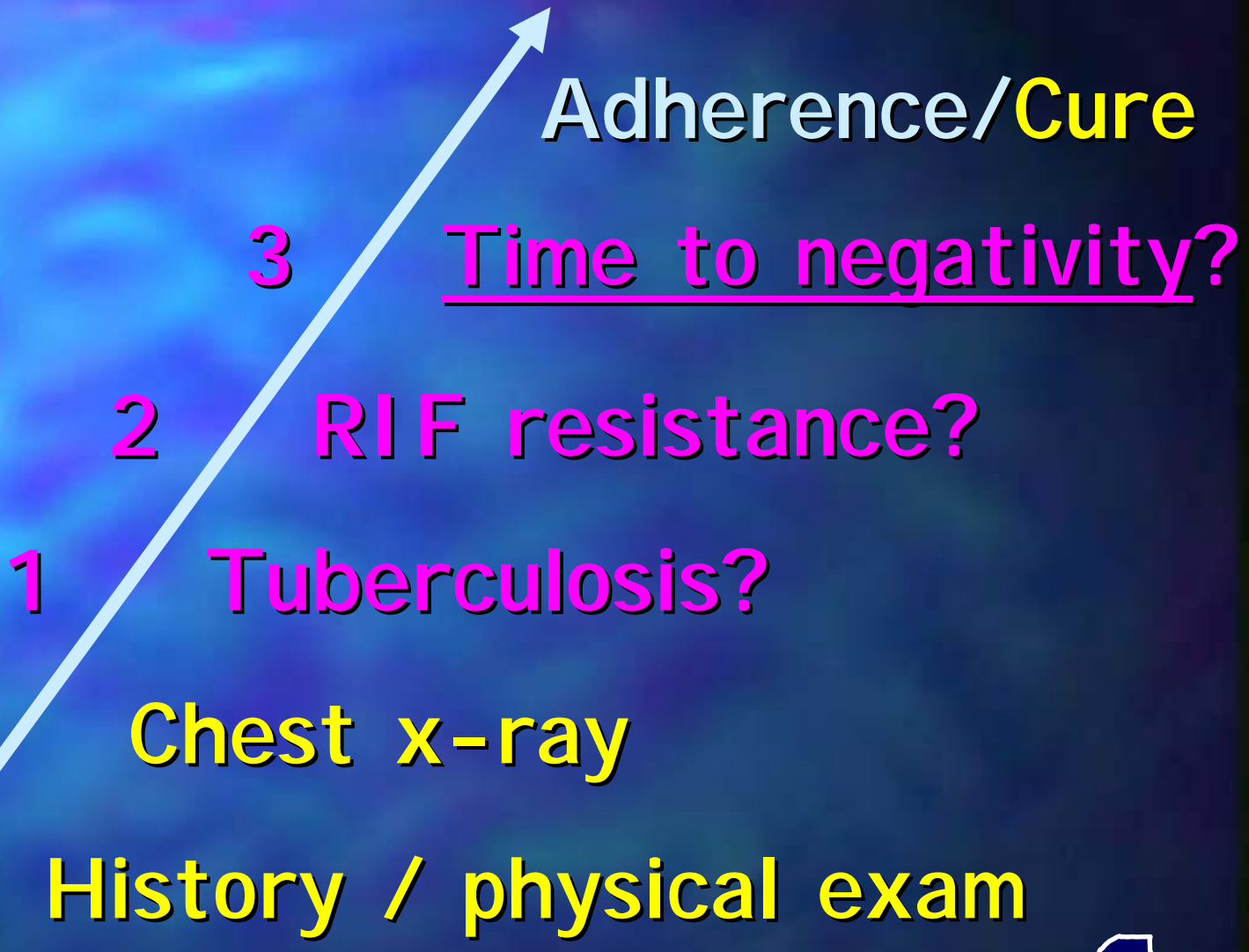


Patient

sees

a

doctor



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 -

HIV +

85% pulmonary TB
15% extrapulmonary TB

33% pulmonary TB
33% extrapulmonary TB
33% both

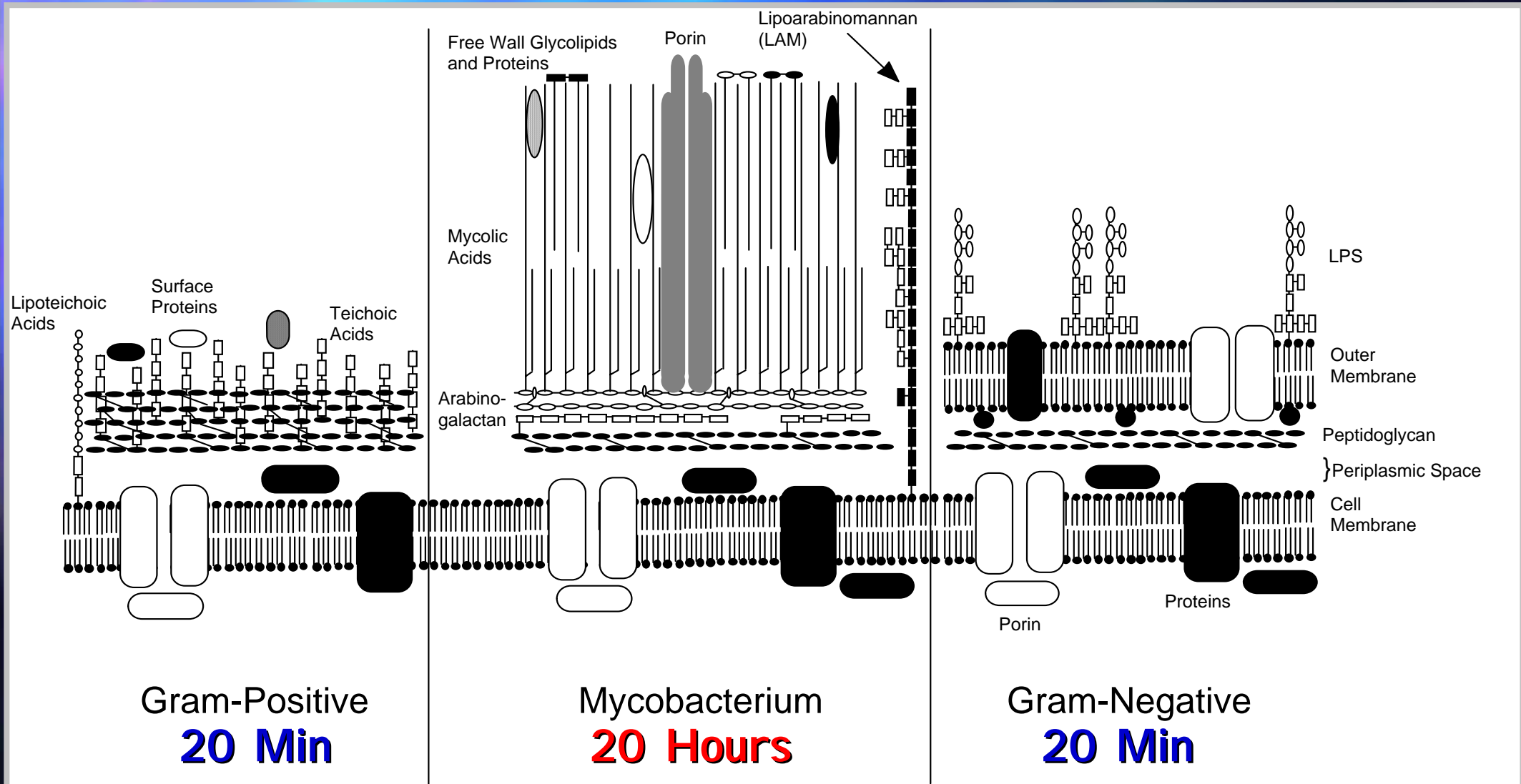


Collection & transport

- 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



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:

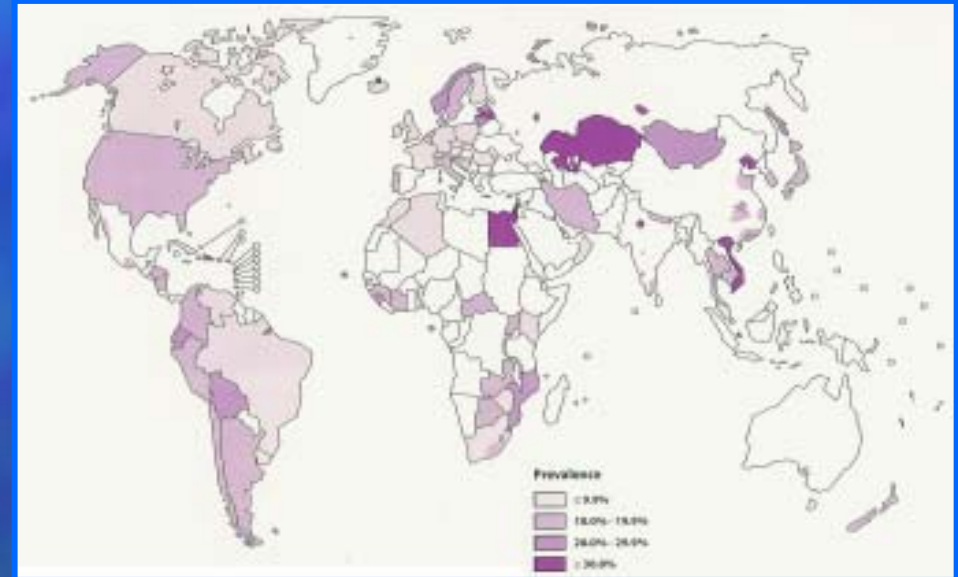
	HIV	TB	MDR
Malawi	79%	11%	0%
Thailand	71%	9%	25%

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



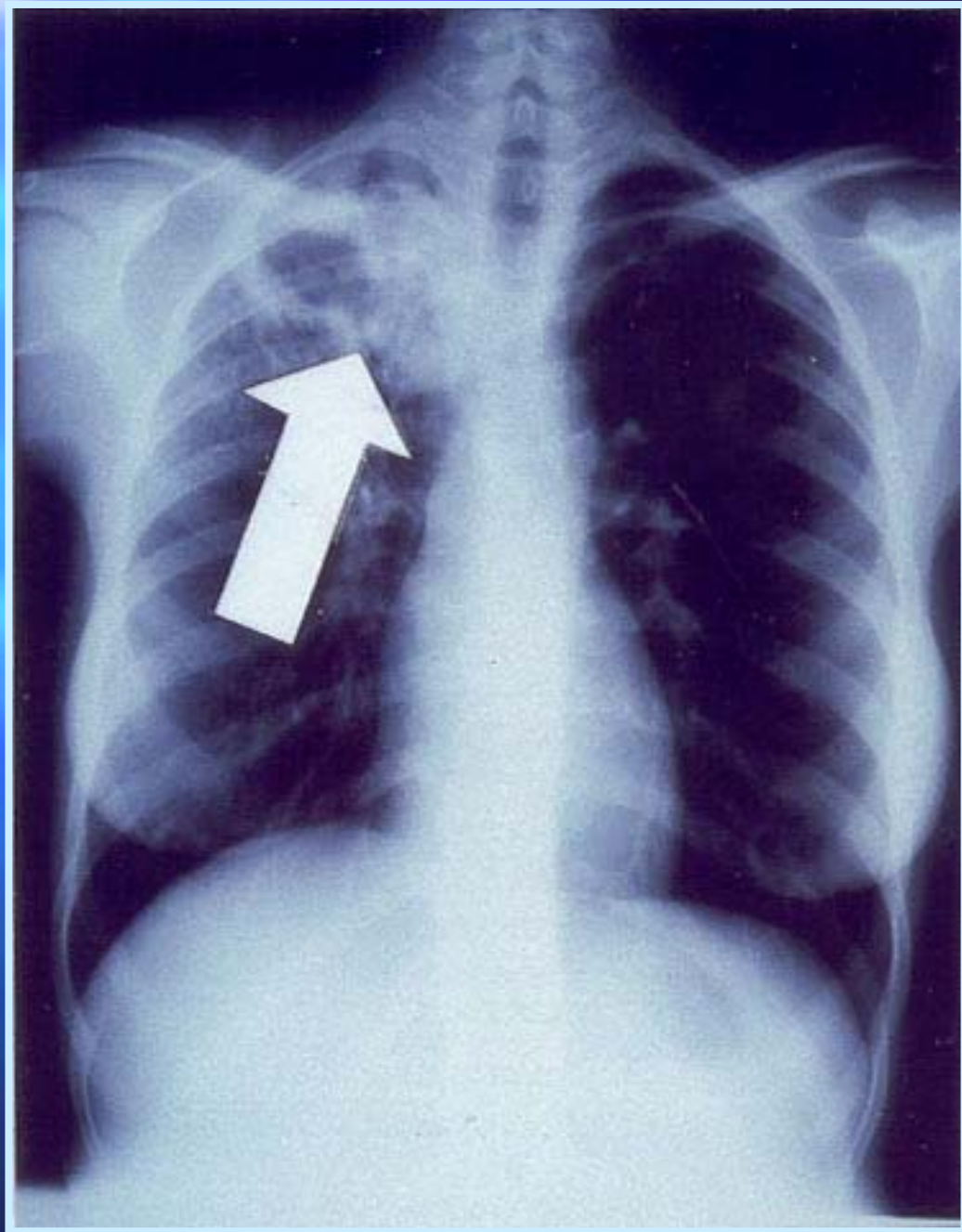
Resistant and MDR TB

- 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
Turnaround
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 and 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



I dentification

- **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

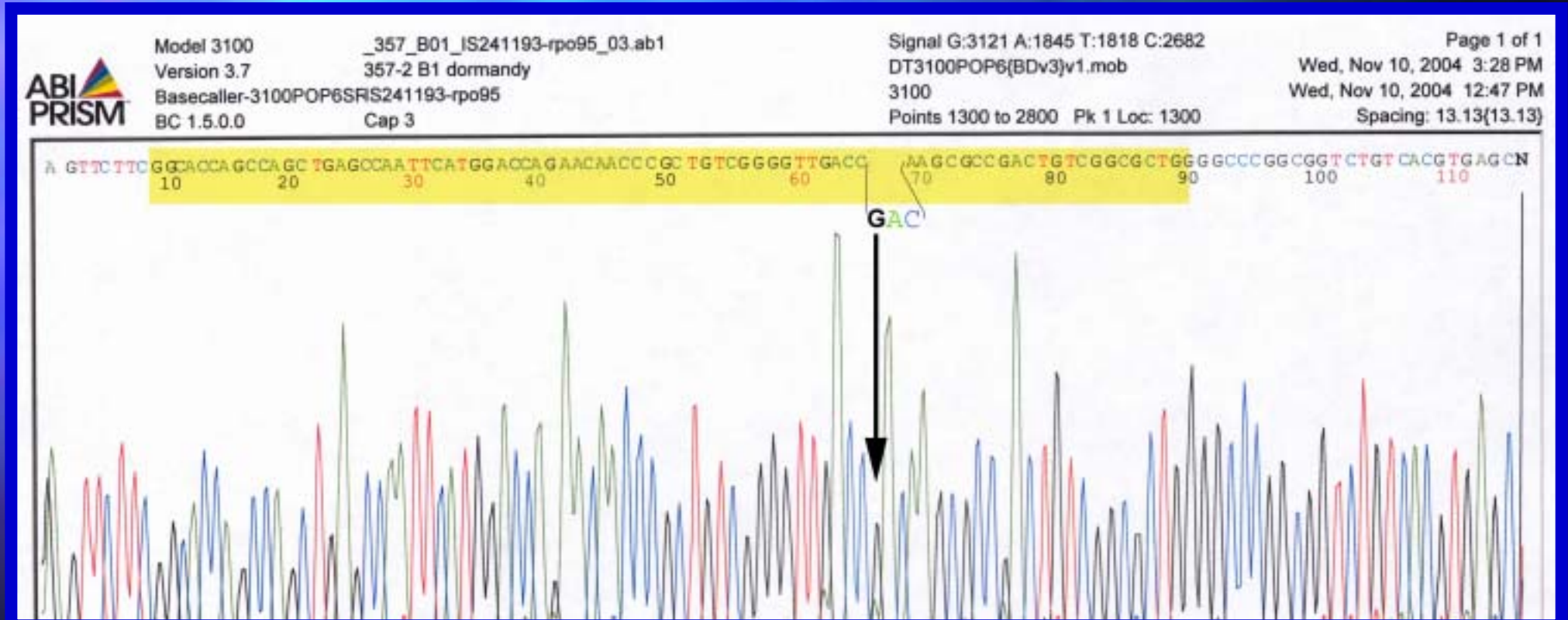
Agar based AST

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
0095-1137/03/\$08.00+0 DOI: 10.1128/JCM.41.7.2822-2826.2003
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Vol. 41, No.

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

Akos Somoskovi,^{1,2} Qunfeng Song,^{1,3} Judit Mester,^{1,4} Charise Tanner,⁵
Yvonne M. Hale,⁵ Linda M. Parsons,^{1,6} and Max Salfinger^{1,6,7*}

Wadsworth Center, New York State Department of Health,¹ Department of Biomedical Sciences, School of Public Health, University at Albany,² and Department of Medicine, Albany Medical College,³ Albany, New York; Department of Respiratory Medicine, School of Medicine, Semmelweis University,⁴ and Korányi National Institute for Tuberculosis and Respiratory Medicine,⁴ Budapest, Hungary; Guizhou Provincial Epidemic Preventive Station, Guiyang, China³; and Bureau of Laboratories, Florida Department of Health, Jacksonville, Florida⁵

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	<i>rpoB</i>	>96%
PZA	<i>pncA</i>	97%
INH	<i>katG</i>	40-60%
INH-ETH	<i>inhA</i>	15-43%
INH	<i>ahpC</i>	10%
INH	<i>kasA</i>	unknown



Drug-Resistant TB -

A Survival Guide For Clinicians

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

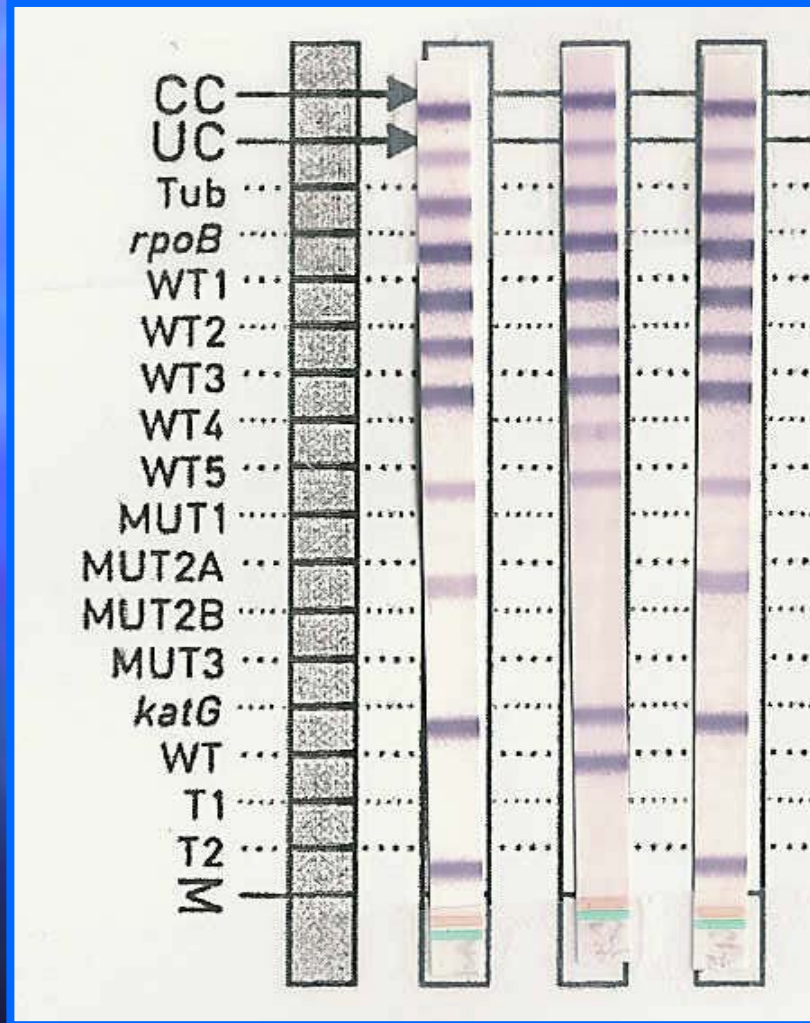
www.nationaltbcenter.edu



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

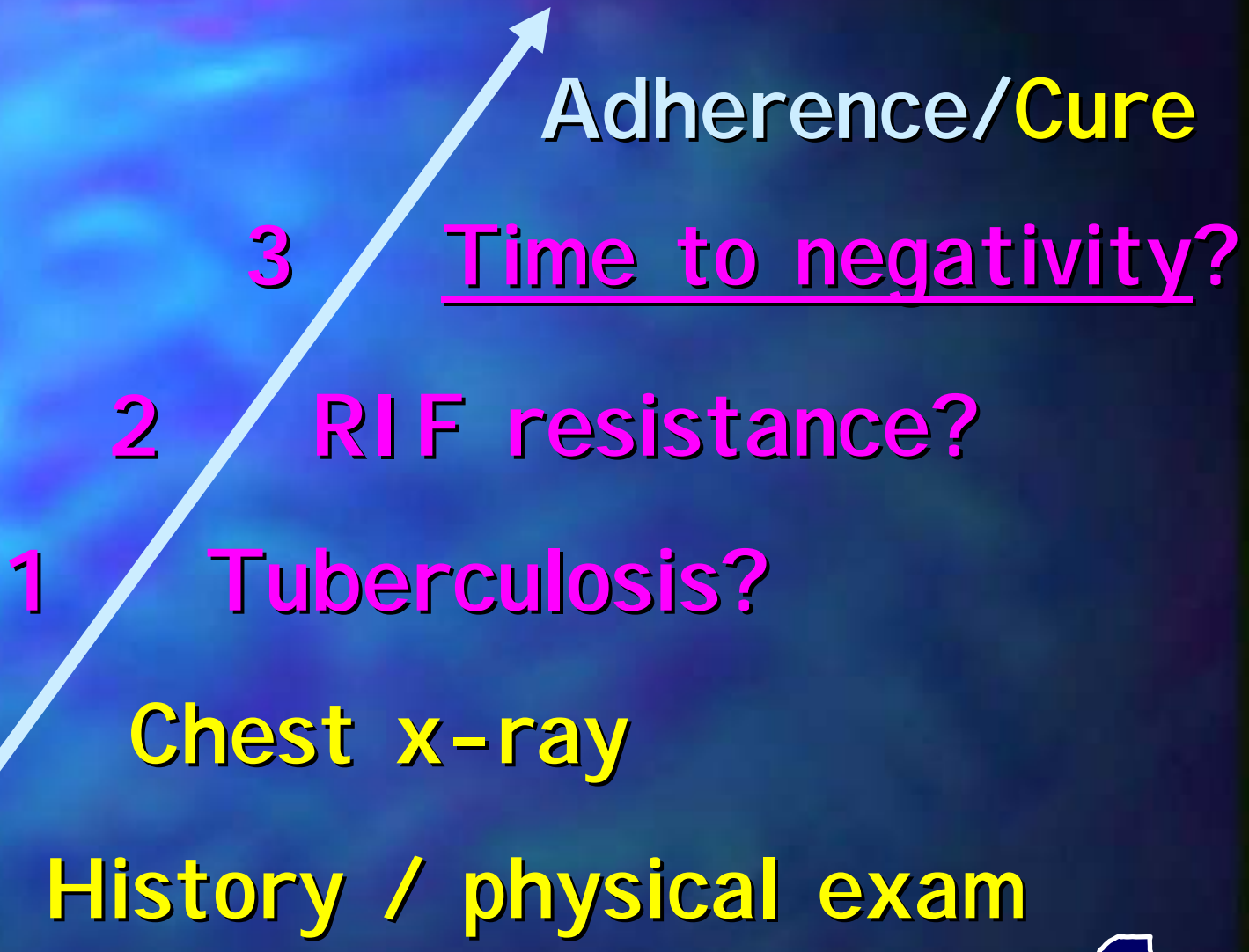


Patient

sees

a

doctor



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 I I

- 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 (I UATLD)

'91: DOTS (WHO)

'99: DOTS-Plus*

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



DOTS PLUS

- I) Second-line drugs availability
- II) Drug resistance results in real time



WHO / IUATLD:

Initial: Sputum microscopy

If **positive**, treat

@ Month 3: Sputum microscopy

If **positive**, then measure and **AST**



THE GLOBAL PLAN
TO STOP TB
2006-2015



Actions for Life

TOWARDS A WORLD FREE OF TUBERCULOSIS

Stop  Partnership

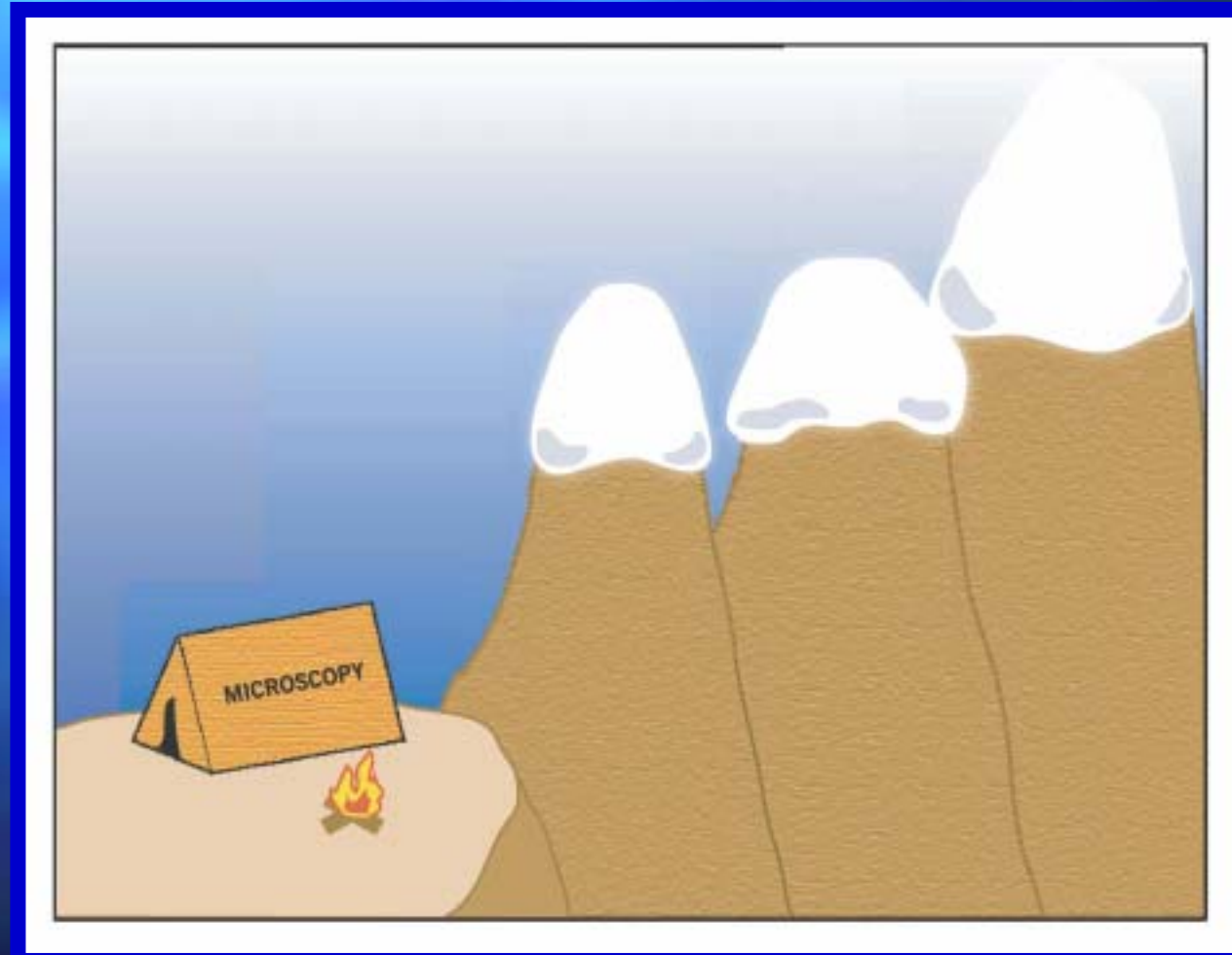


Actions for life towards a world free of tuberculosis:

- 1) Will expand equitable access for all to quality TB diagnosis and treatment



Is like conquering the Himalayan mountains



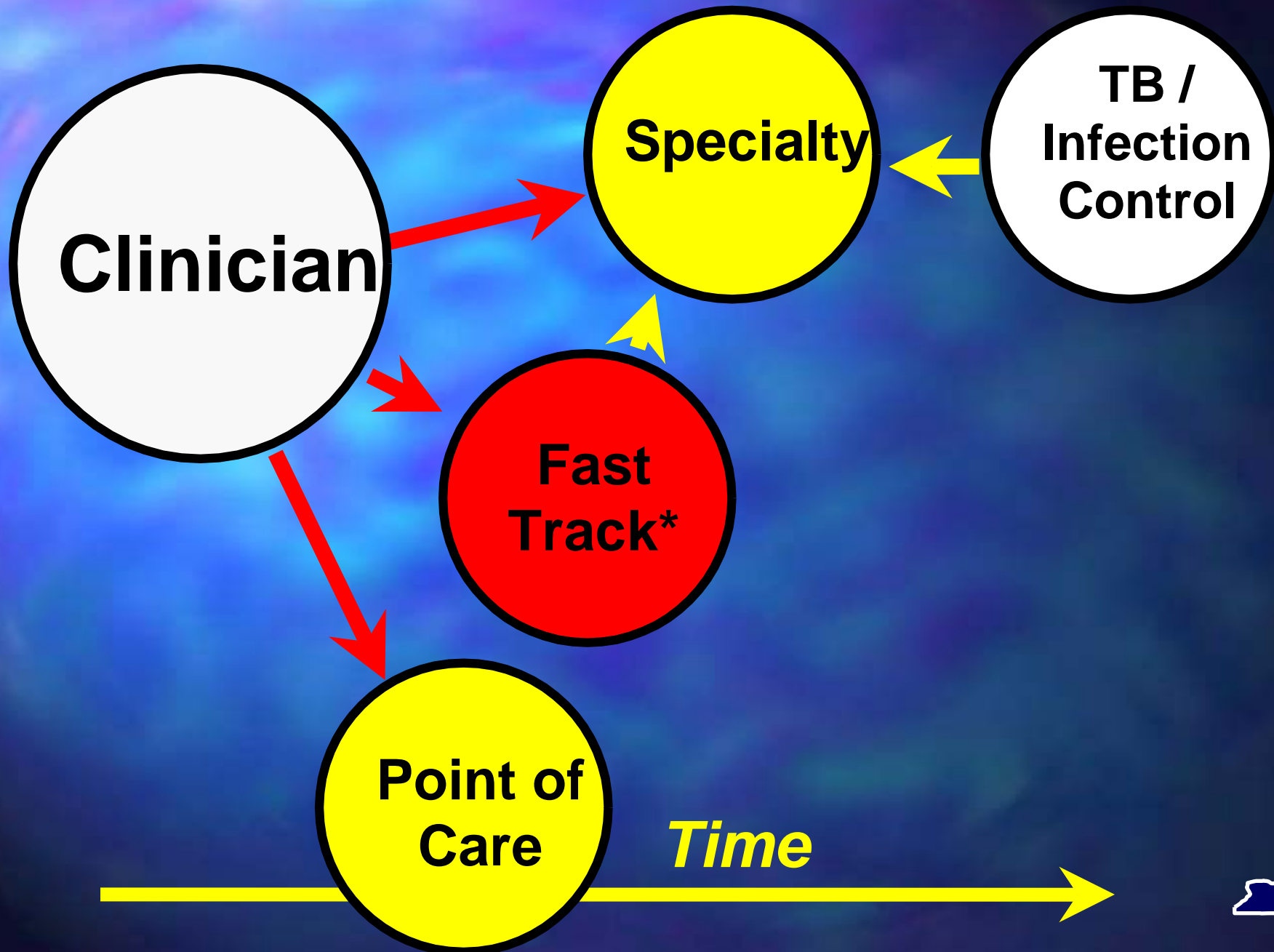


Requires additional resources!



In closing, a
paradigm
shift is
warranted





*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!



Never Give Up!



- Fighting TB
- Fighting poverty
- Standing up for

PEACE On Earth!



Obrigadoh!

Gracias!

Merci!

Danke!

Thank you!

Tesekkür
ederim!

Spacibo!

Grazie!

