WEBINAR:

The Race Is On: Faster Turnaround Times in the Diagnosis of Multidrug-Resistant Tuberculosis

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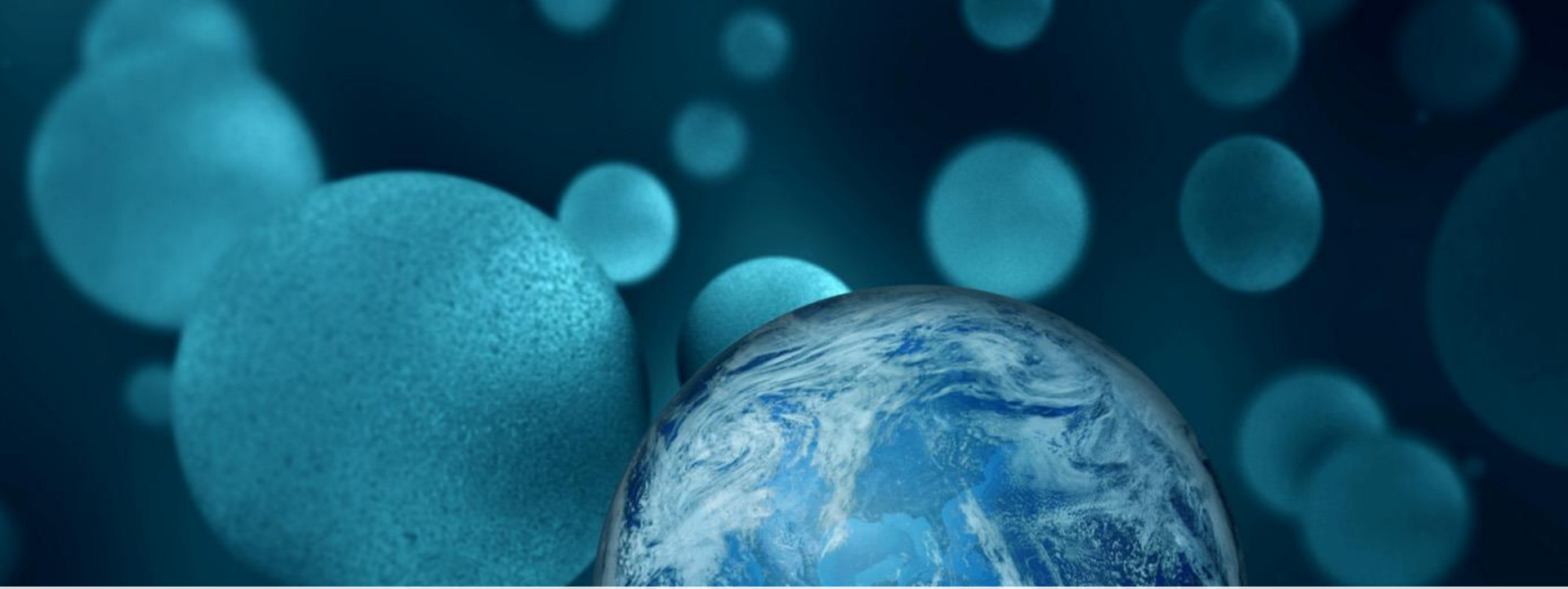
National Jewish Health
Denver, Colorado

December 10, 2015



ThermoFisher SCIENTIFIC

The Race Is On: Faster Turnaround Times in the Diagnosis of Multidrug-Resistant Tuberculosis

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Conflicts of Interest

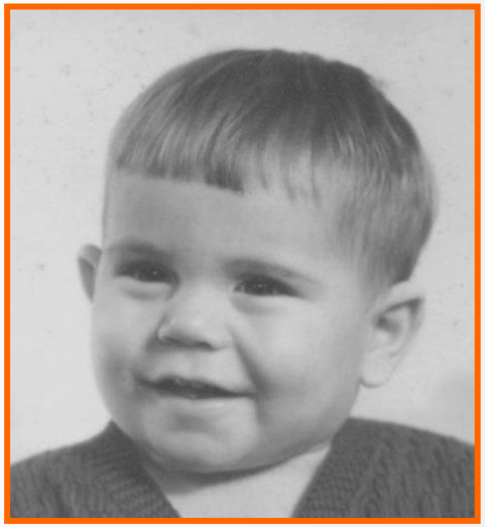
- *Any views or opinions expressed in this webinar are solely that of the presenter and do not necessarily represent those of the sponsor, Thermo Fisher Scientific, or Current Protocols.*

Topics

- Introduction
- Epidemiology – USA and global
- 3 important questions
- Systems thinking

The journey sets the destination

- 1978-1981 University Hospital, Basel-Switzerland
- 1981-1992 University of Zurich, Dept. Medical Microbiology
- 1986-1988 Sabbatical – Denver, Colorado
  National Jewish, University Hospital,
  Webb-Waring Lung Institute
- 1992-2006 New York State DOH, Albany, New York
- 2006-2012 Florida DOH, Tallahassee, Florida
- October 2012 National Jewish Health, Denver, Colorado



LTBI vs. TB Disease

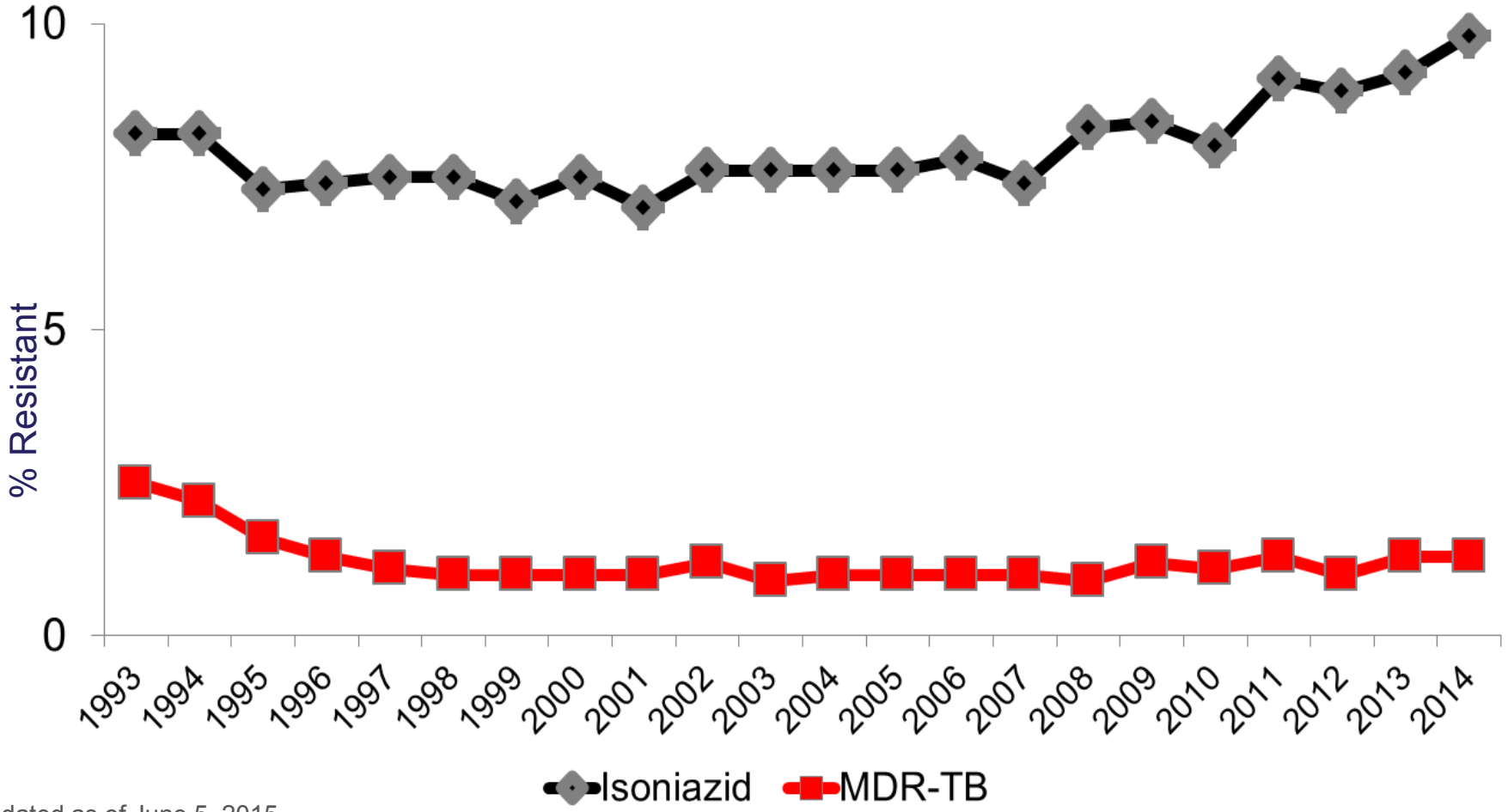
Person with LTBI (Infected)	Person with TB Disease (Infectious)
Has a small amount of TB bacteria in his/her body that are alive, but inactive	Has a large amount of active TB bacteria in his/her body
Cannot spread TB bacteria to others	May spread TB bacteria to others
Does not feel sick, but may become sick if the bacteria become active in his/her body	May feel sick and may have symptoms such as a cough, fever, and/or weight loss
Usually has a TB skin test or TB blood test reaction indicating TB infection	Usually has a TB skin test or TB blood test reaction indicating TB infection
Radiograph is typically normal	Radiograph may be abnormal
Sputum smears and cultures are negative	Sputum smears and cultures may be positive
Should consider treatment for LTBI to prevent TB disease	Needs treatment for TB disease
Does not require respiratory isolation (AII)	May require respiratory isolation (AII)
Not a TB case	A TB case

Topics

- Introduction
- **Epidemiology – USA and global**
- 3 important questions
- Systems thinking

7

Primary Anti-TB Drug Resistance, United States, 1993 – 2014*

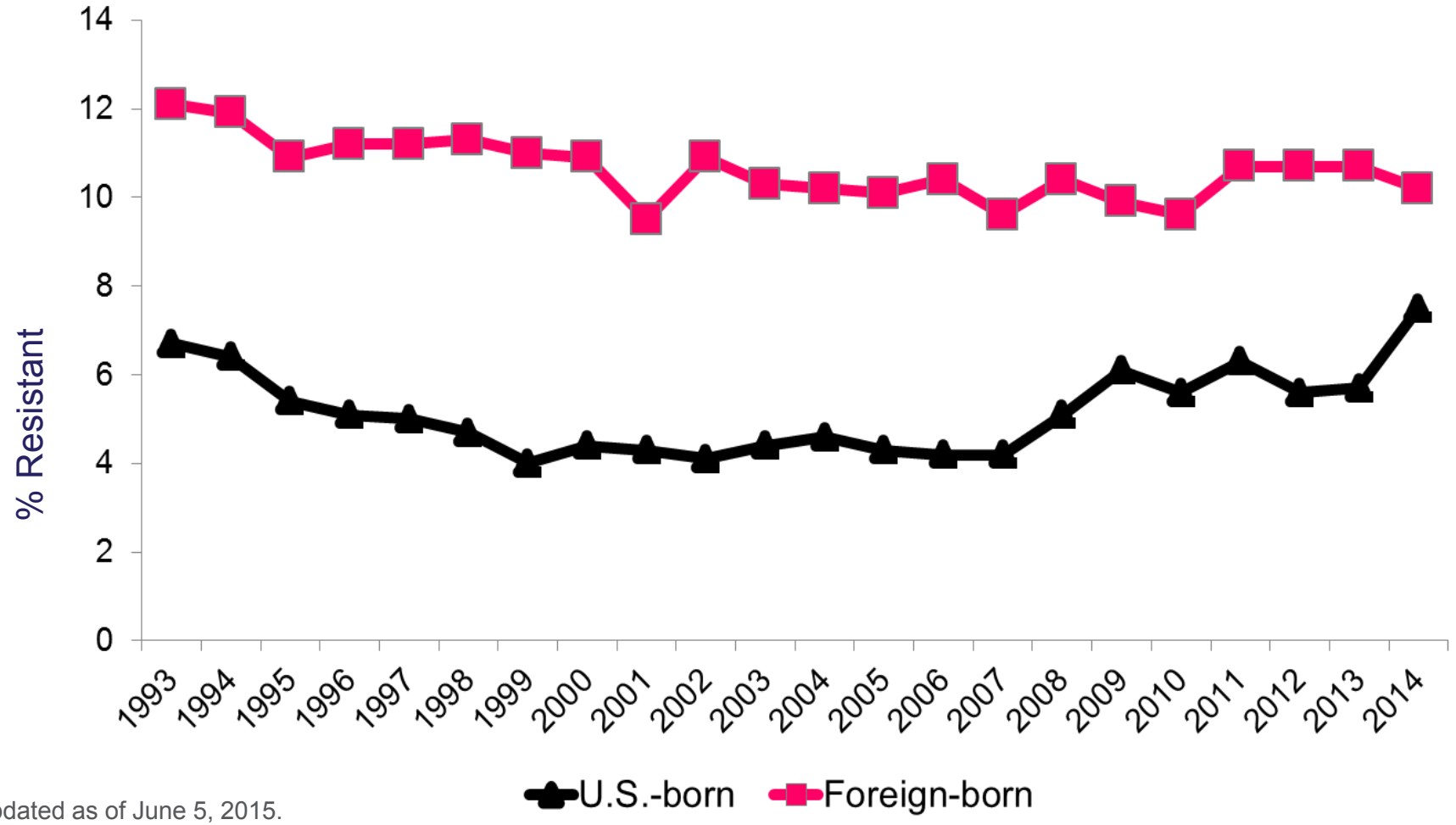


*Updated as of June 5, 2015.

Note: Based on initial isolates from persons with no prior history of TB. Multidrug resistant TB (MDR TB) is defined as resistance to at least isoniazid and rifampin.

Source: CDC

Primary Isoniazid Resistance in U.S.-born vs. Foreign-born Persons, United States, 1993 – 2014*

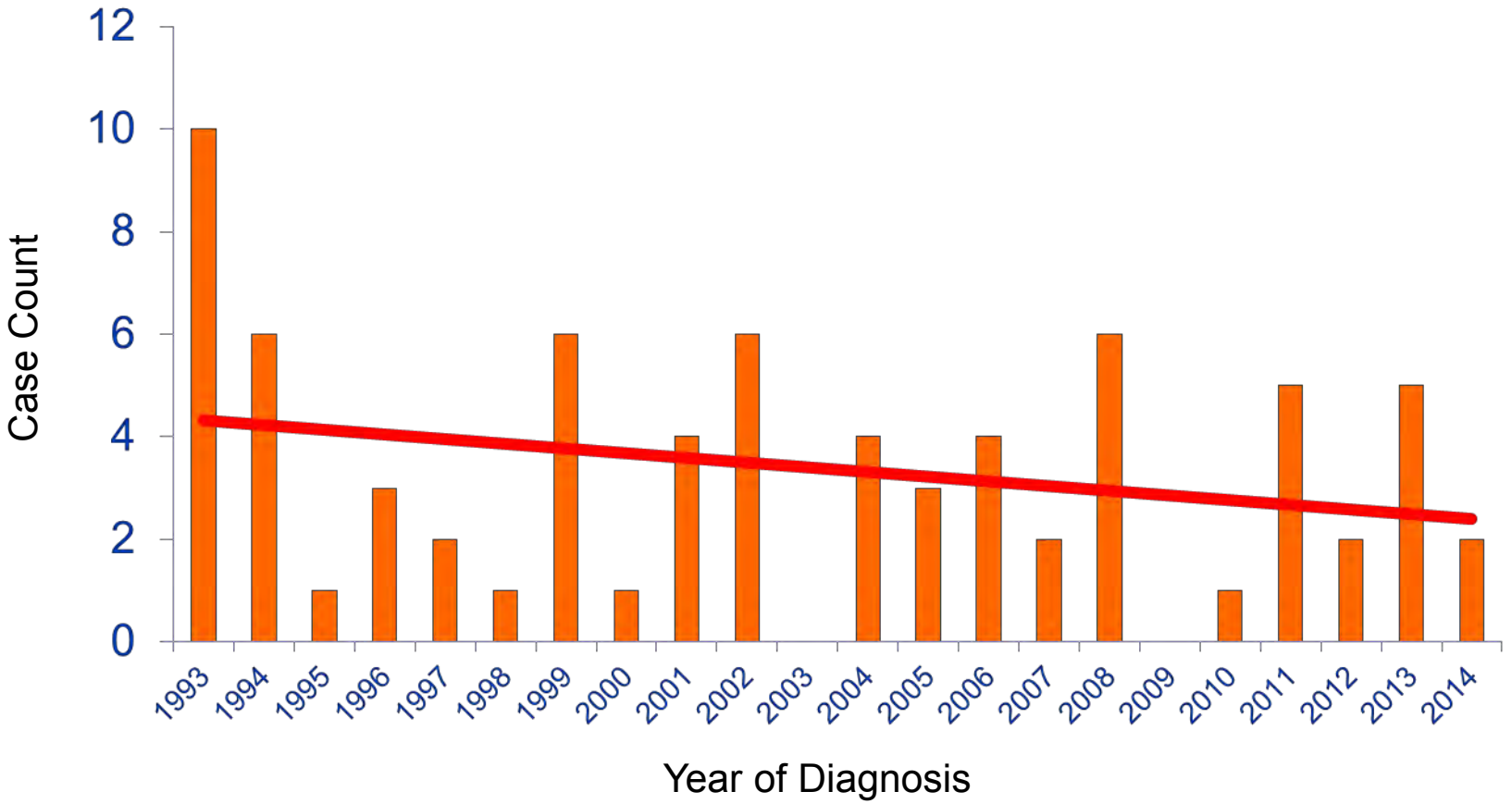


*Updated as of June 5, 2015.

Note: Based on initial isolates from persons with no prior history of TB.

Source: CDC

XDR TB Case Count Defined on Initial AST* by Year, 1993 – 2014**



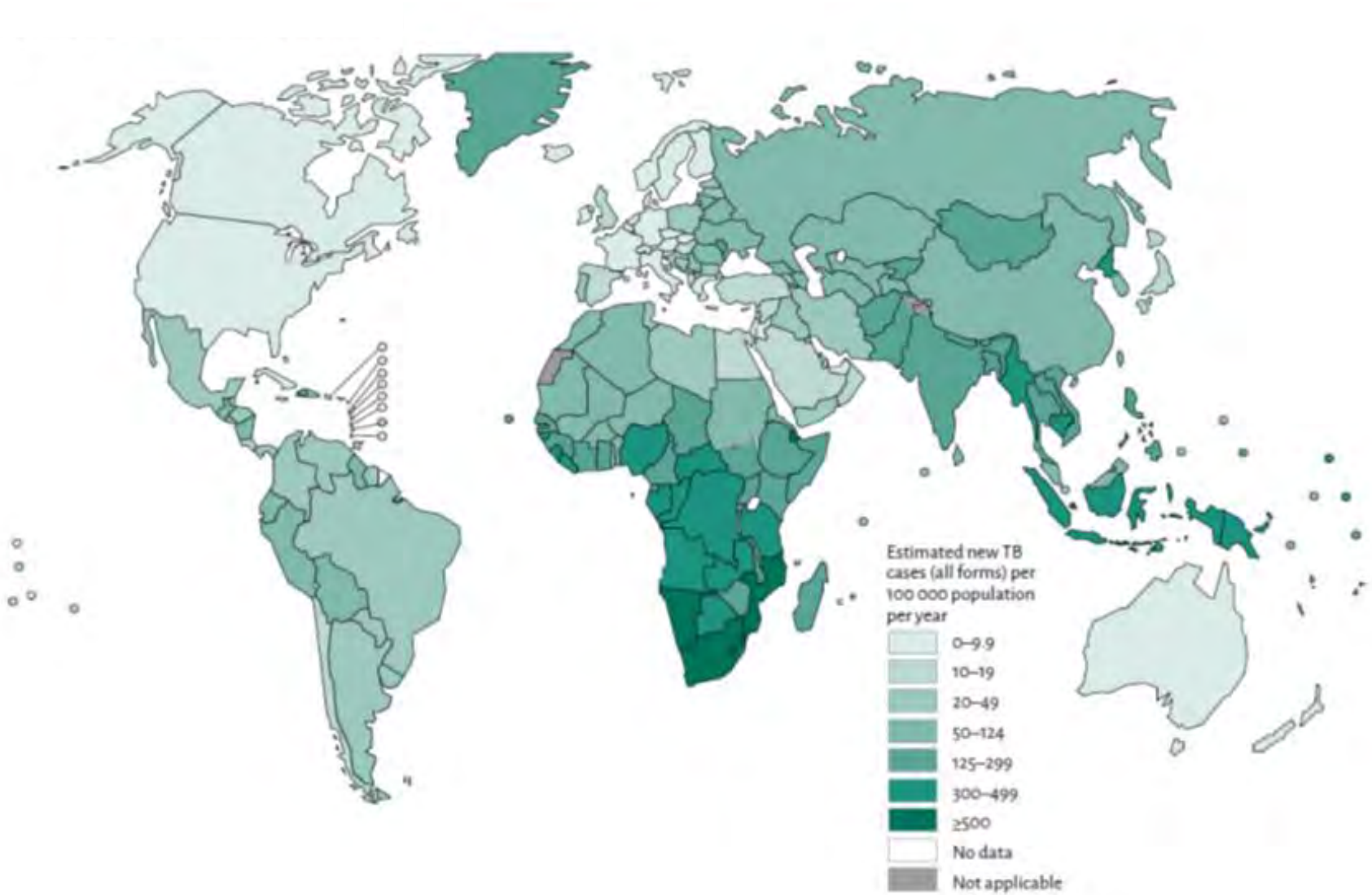
* Antimicrobial susceptibility test.
** Updated as of June 5, 2015.
Note: Extensively drug-resistant TB (XDR TB) is defined as resistance to isoniazid and rifampin, plus resistance to any fluoroquinolone and at least one of three injectable second-line anti-TB drugs.
.Source: CDC

Marshall McLuhan, 1911-1980

WHO Global TB Report 2015

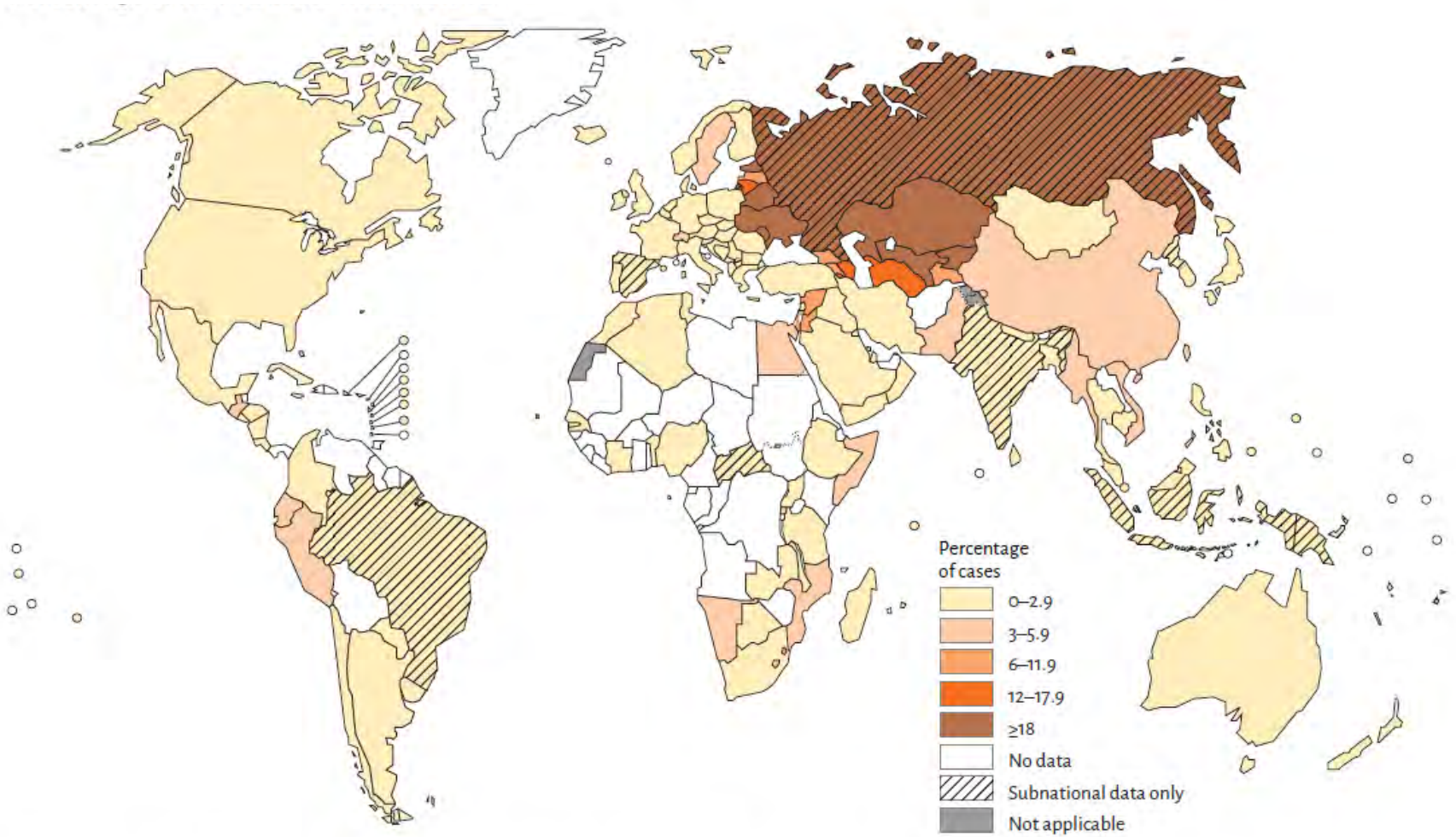
- **Effective diagnosis and treatment saved 43 million lives between 2000-2014**
- **In 2014:**
  - 1.5 million people died of TB
  - 9.6 million people fell ill with TB
  - Of the 480,000 estimated MDR-TB, only 123,000 detected
- **Funding gap for 2015:**
  - Implementing existing interventions- **$1.4 billion**
  - Research and development- **$1.3 billion**

Estimated TB incidence rates, 2014



Graphs can be found in Global Tuberculosis Report 2015, 20th Edition, World Health Organization

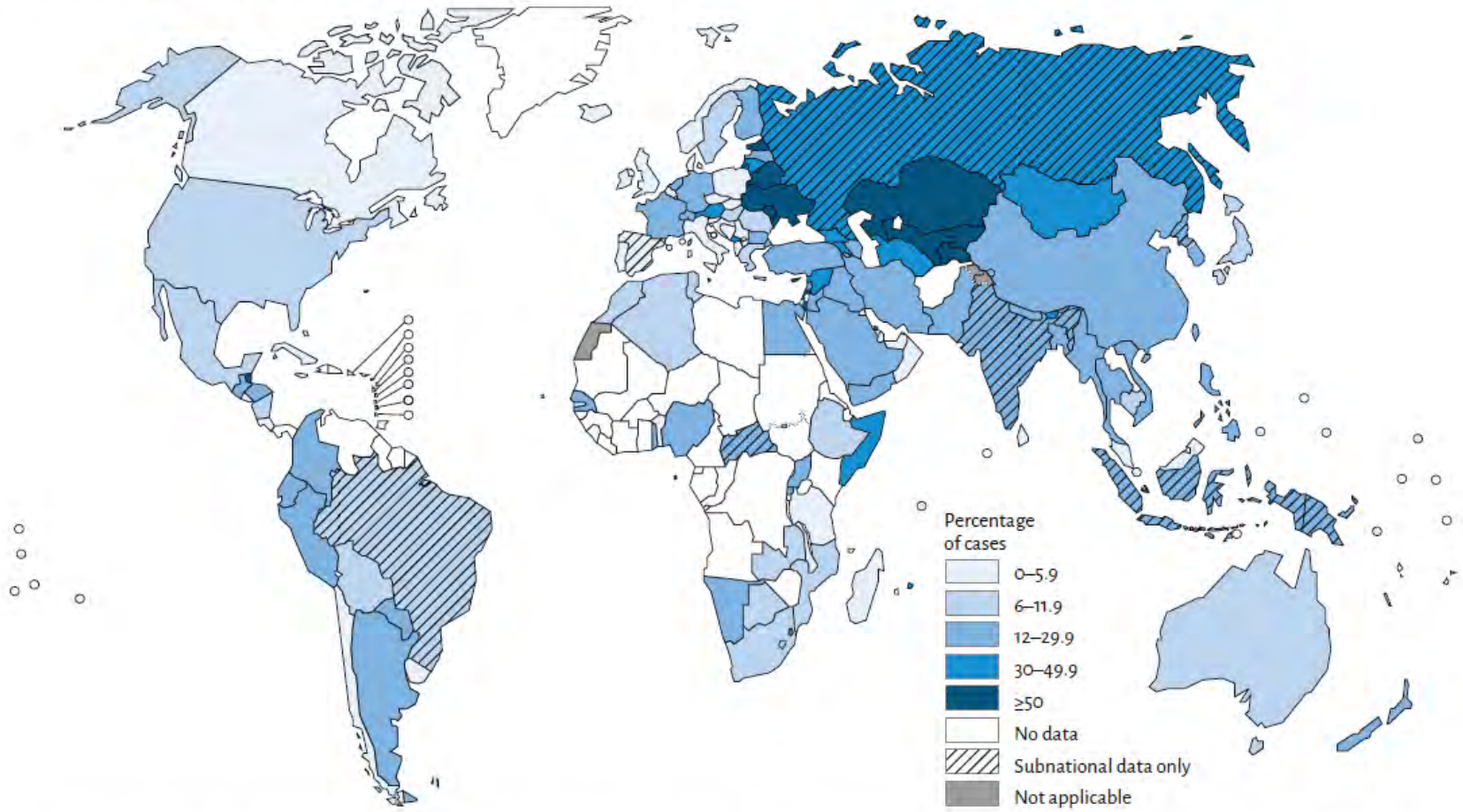
Percentage of new TB cases with MDR-TB*



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before the year 2000 are not shown.

Graphs can be found in Global Tuberculosis Report 2015, 20th Edition, World Health Organization

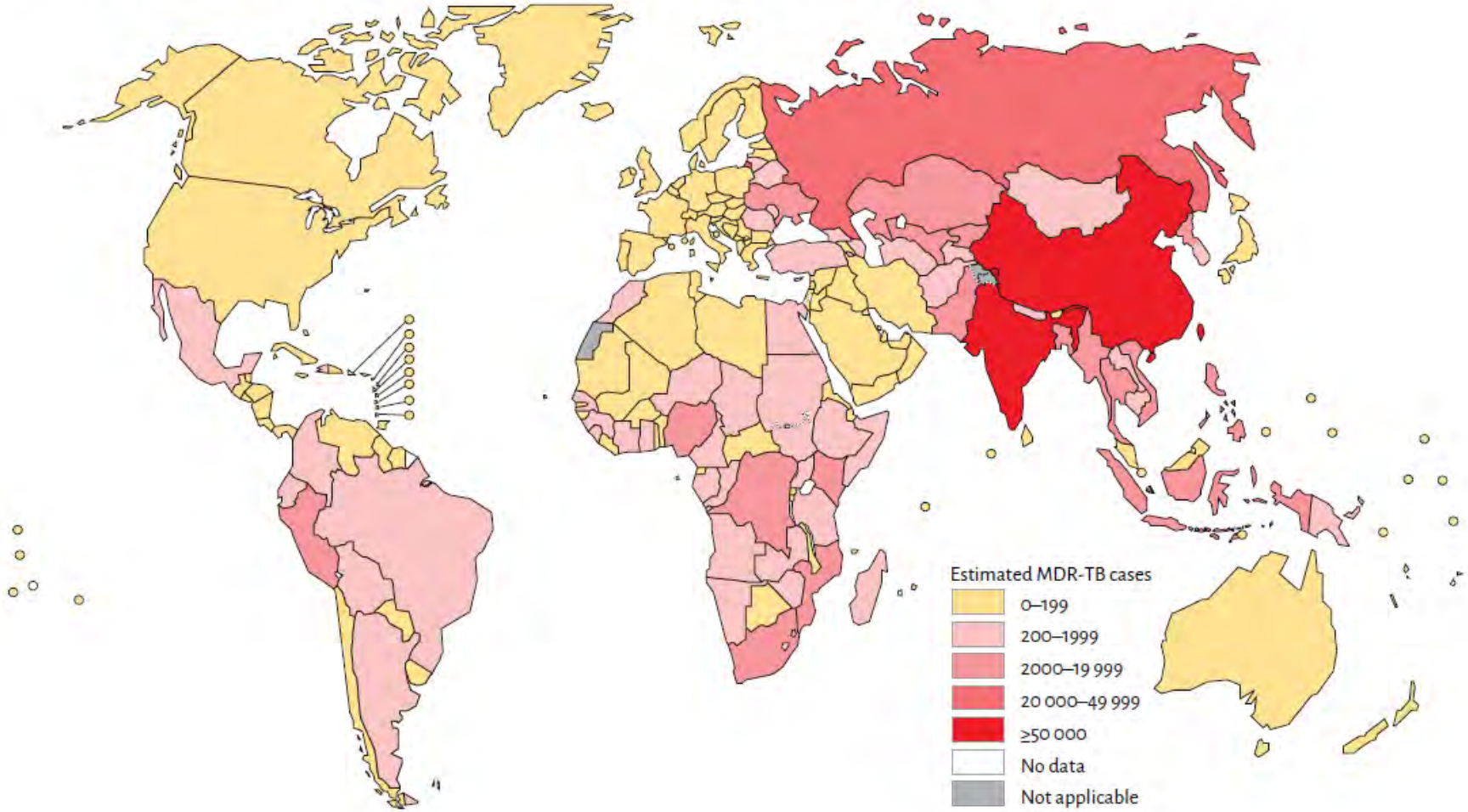
Percentage of previously treated TB cases with MDR-TB*



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before the year 2000 are not shown. In six countries or territories, the high percentages of previously treated cases with MDR-TB refer to only a small number (1–8) of notified TB cases. These are: Bahrain; Belize; Bonaire, Saint Eustatius and Saba; Cyprus; Israel; and Sao Tomé and Principe.

Graphs can be found in Global Tuberculosis Report 2015, 20th Edition, World Health Organization

Number of MDR-TB cases estimated to occur among notified pulmonary TB cases, 2014

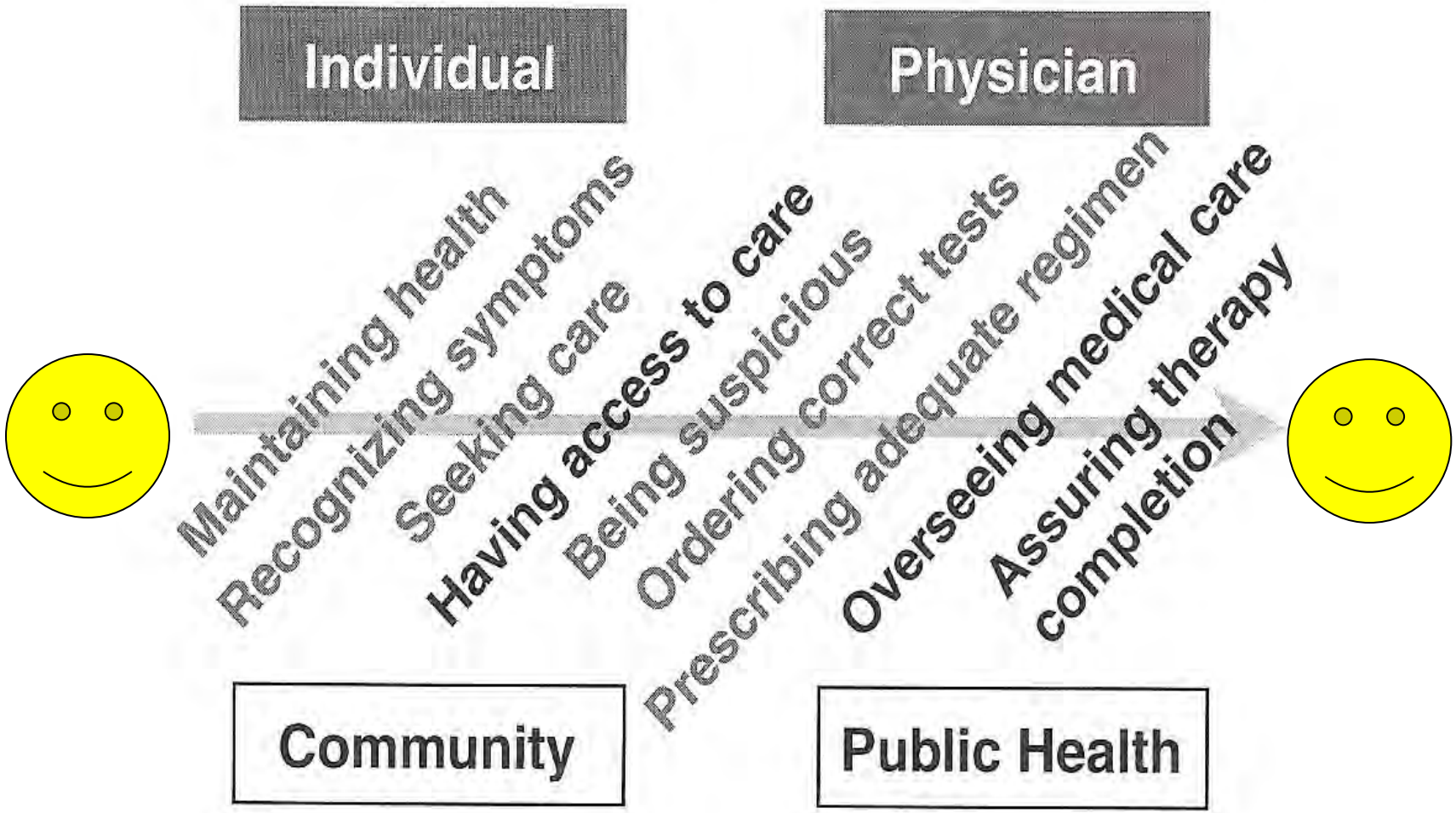


Graphs can be found in Global Tuberculosis Report 2015, 20th Edition, World Health Organization

Topics

- Introduction
- Epidemiology – USA and global
- **3 important questions**
- Systems thinking

Promoting healthy outcomes!



Parsons et al. Infectious Disease Clinics of North America 11:905-928 (1997)

Differential diagnosis?

Three important questions for the laboratory

Photo approved by patient

Sees a doctor

History / physical exam

Chest X-ray

1 Tuberculosis?

2 RIF resistance?

3 Time to negativity?

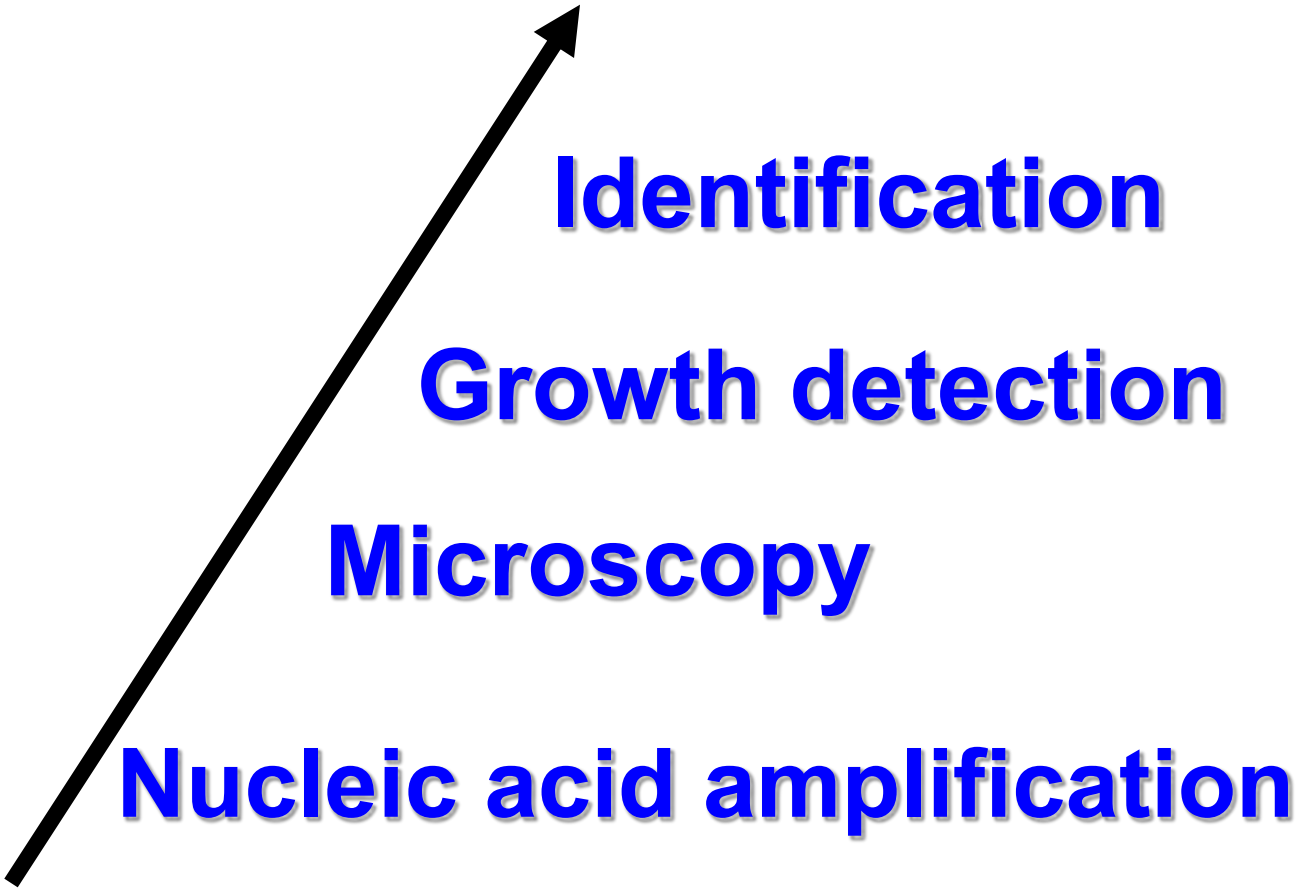
Adherence/Cure

1. Tuberculosis

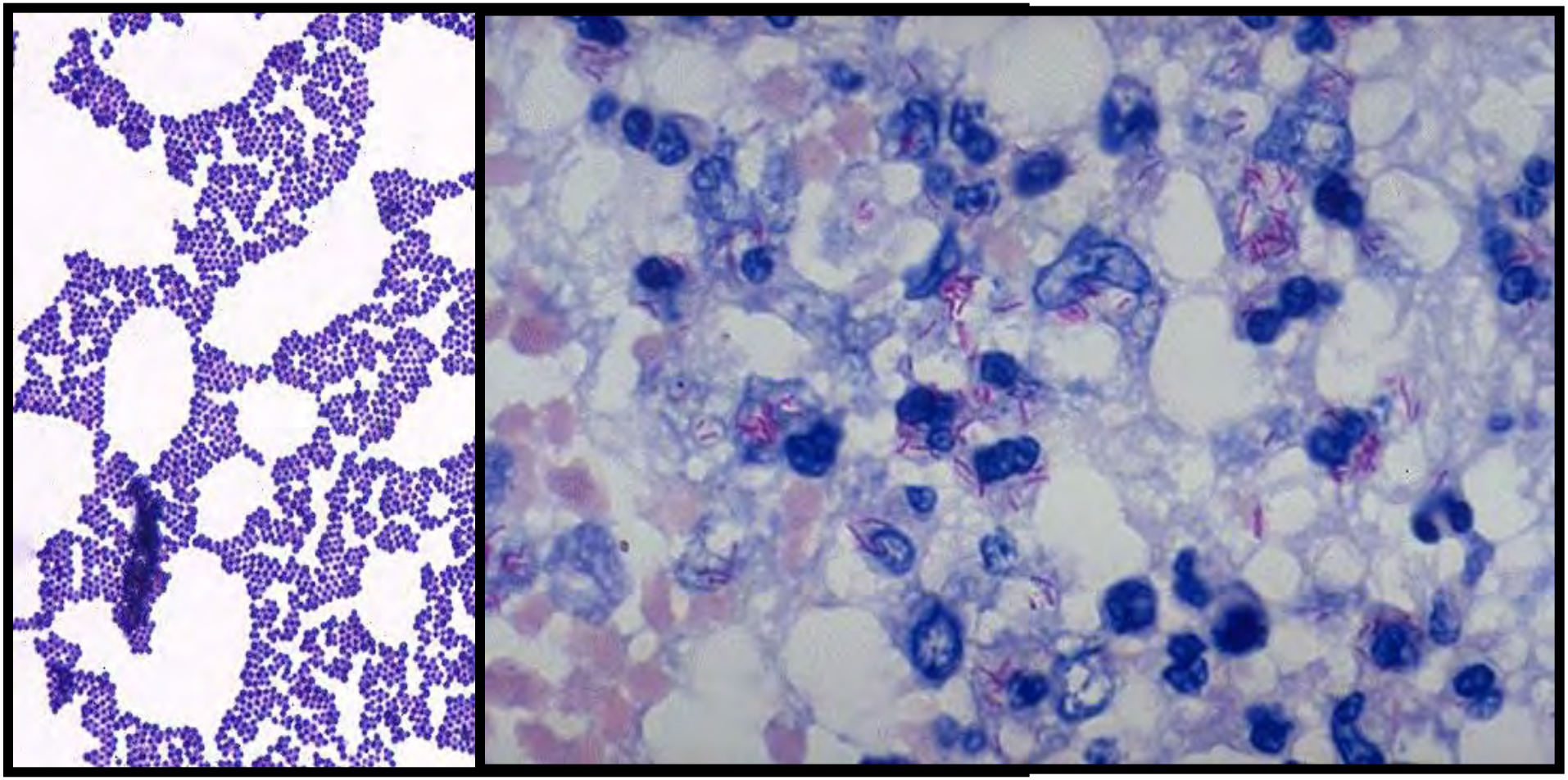
yes

or

no?



Demanding instant results!



20 Min **20 Hours**

Mycobacteriology methods

Microscopy & Growth Detection

1883 **Ziehl-Nielsen** - Carbol Fuchsin (hot) staining
1909 **Uhlenhuth / Kinyoun** - Carbol Fuchsin (cold) staining
1938 **Hagemann** – Fluorescence Auramine staining

1900's **Guinea pig** (paucibacillary specimens**)**
1932 **Loewenstein-Jensen** – Egg-based solid medium
1946 **Dubos-Davis** – Liquid medium
1947 **Dubos-Middlebrook** – Agar-based solid medium
1977 **Middlebrook et al** – Radiometric broth medium
1983 **Roberts et al** – Radiometric drug susceptibility testing
1999 **Hanna et al** – Walk-away system

Molecular TB Assays

1987 **DNA Probes for identification**
1991 **Cave et al** – IS*6110* for fingerprinting
1991 **Eisenach et al** – PCR from sputum
1992 **Boettger et al** – *Mycobacterium genavense*
1993 **Telenti et al** – *rpoB* sequencing
2006 **Somoskovi et al** – MDR screen in AFB+ sputum
2010 **Helb et al** – Fully integrated sample processing

Nucleic acid amplification TBC

- FDA-approved for respiratory specimens
  - Smear-positive (Dec. ‘95)
  - Smear-negative* (Sept. ‘99)
- MMWR, January 16, 2009 [Universal]

- In July 2013, the FDA granted Market Clearance to the Cepheid Xpert® MTB/RIF assay. This NAA test can simultaneously identify *Mycobacterium tuberculosis* complex (TBC) and genetic mutations associated with resistance to rifampin from raw sputum and concentrated sputum sediments.

NAAT recommendations

"NAA testing should 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."

-MMWR Jan 16, 2009

Sensitivity (%)

	AFB Smear +	AFB Smear -
MTD*	97	76
Laboratory-developed test**	99.6	75.4
Xpert***	100	71.7

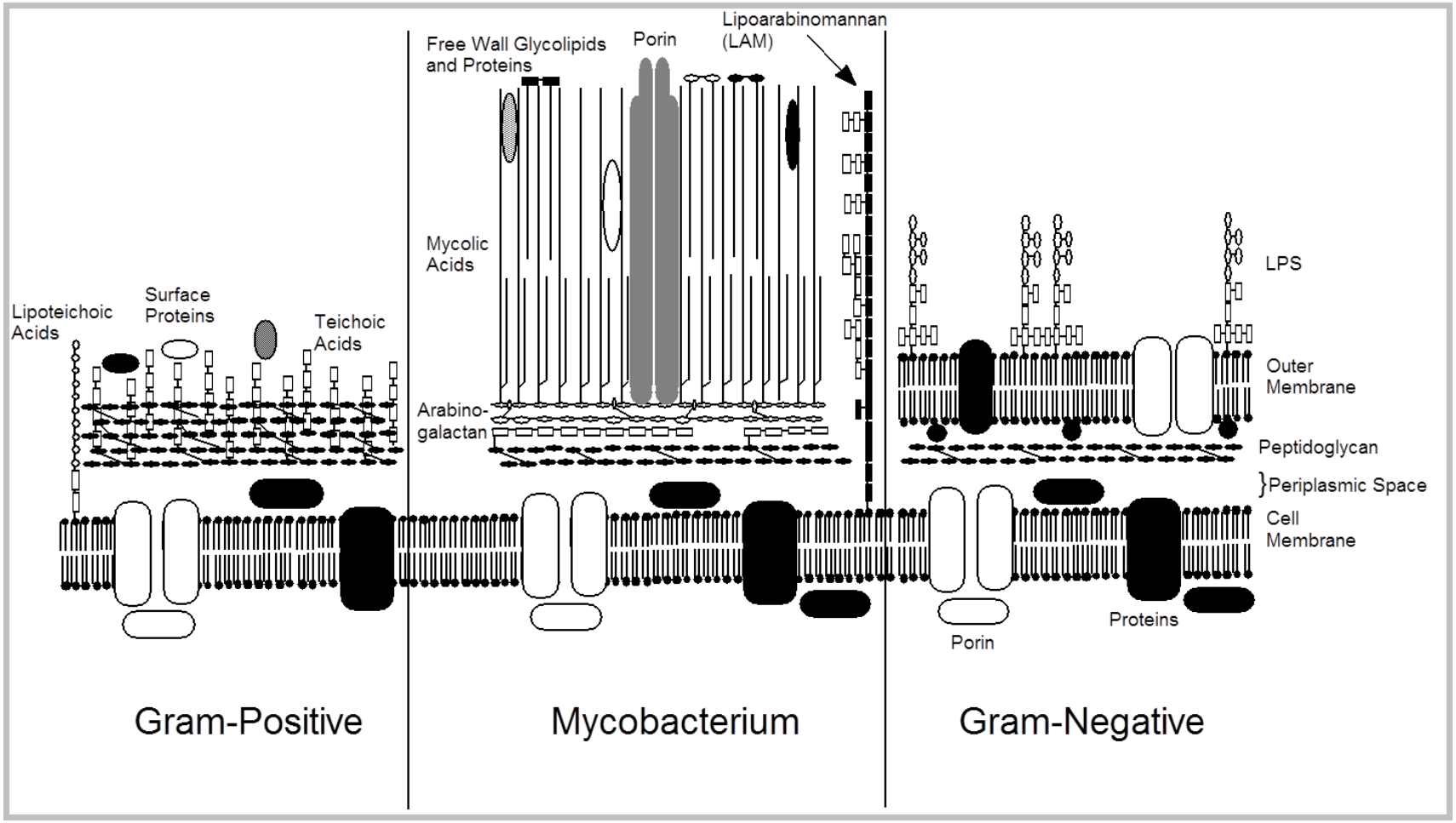
*Greco et al Thorax 61:783-790(2006)
**Halse et al JCM 48:1182-1188(2010)
***Helb et al JCM 48:229-237(2010)

Healthy People 2020

- **IID-32: Increase the proportion of culture-confirmed TB patients with a positive nucleic acid amplification test (NAAT); result reported within 2 days of specimen collection**
- Baseline: **32.0 percent** of culture-confirmed TB patients with a positive NAAT had their test results reported within 2 days of specimen collection in 2008
- Target: **77.0 percent**
- Target-Setting Method: Maintain consistency with national programs, regulations, policies, and laws

Data Source: National TB Surveillance System (TB), CDC/NCHHSTP

Envelopes Gram+, Gram-, Mycobacterium



Parsons, L.M., et al. 1997 Infect. Dis. Clinics N.A. *11*:905-928

Reading/Interpretation: ZN & F Stain

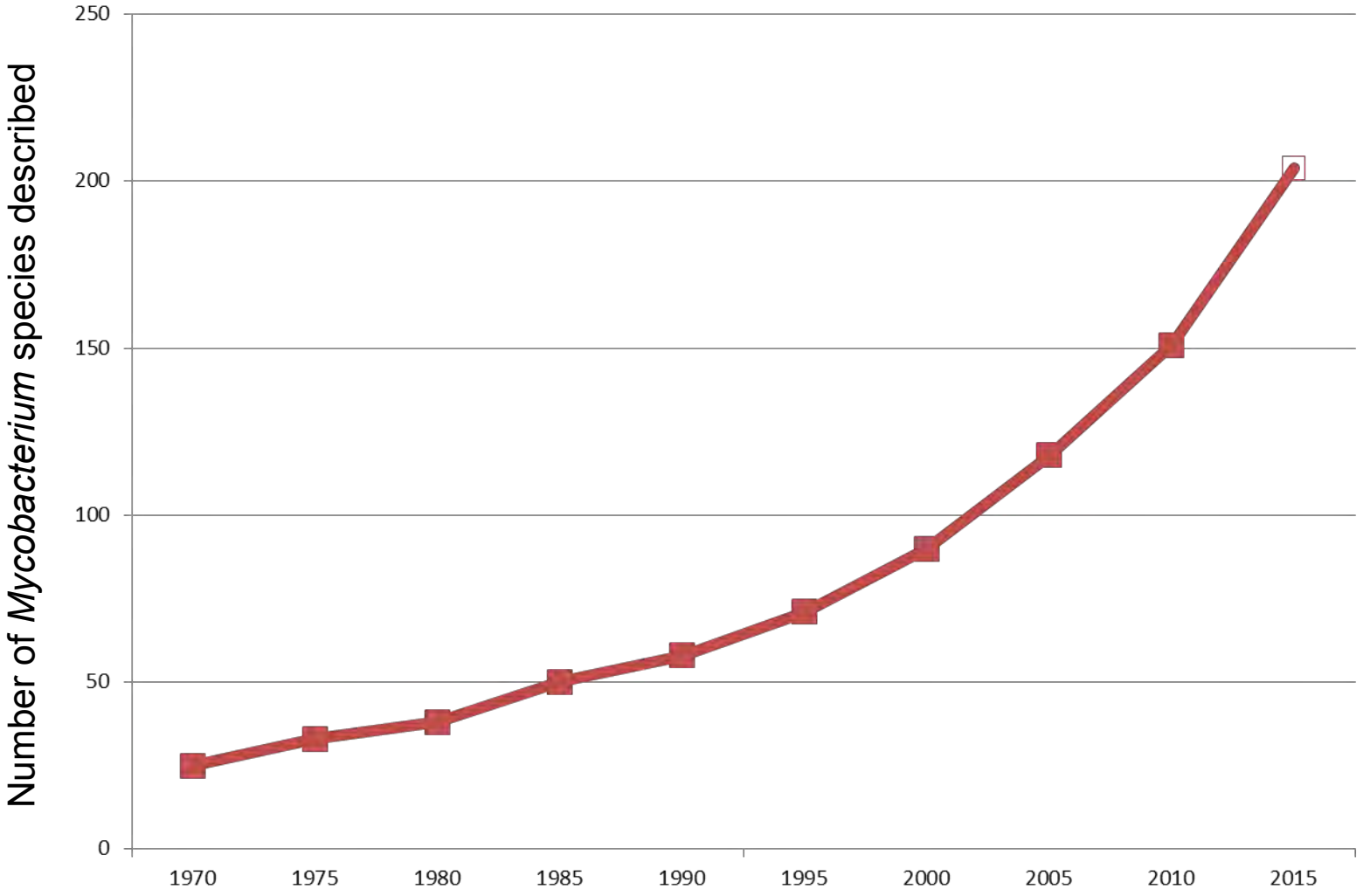
AFB Number per view fields (1000 X oil immersion)	AFB Number per view fields (250 X)	
None per 300 fields	None per 30 fields	No AFB seen
1-2 per 300 fields	1-2 per 30 fields	Doubtful, repeat
1-9 per 100 fields	1-9 per 10 fields	Rare, 1+
1-9 per 10 fields	1-9 per field	Few, 2+
1-9 per field	10-90 per field	Moderate, 3+
>9 per field	>90 per field	Numerous, 4+

A quantification of the numbers of acid-fast organisms per field should be rated 1+ to 4+. The number of tubercle bacilli in pulmonary secretions is directly related to the risk of transmission.

Processing sputum

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

Mycobacterium sp.



Source: http://www.bacterio.net/mycobacterium.html

Mycobacterium

- **172** Species and **13** Subspecies in genus *Mycobacterium* as of November 2015
- ***M. tuberculosis* complex**
  - *M. tuberculosis*
  - *M. bovis*
  - *M. bovis* BCG
  - *M. africanum*
  - *M. caprae*
  - *M. microti*
  - *M. canettii*
  - *M. pinnipedii*
  - *M. mungi*
  - *M. orygis*

M. tuberculosis complex (2001-2004)

	Number	Percent
M. tuberculosis	1,594	94.6%
M. africanum	31	1.8%
M. bovis	36	2.1%
M. caprae	1	0.1%
***M. bovis* BCG**	23	1.4%

Wadsworth Center – NYS-DOH

Rapid and Simple Approach for Identification of *Mycobacterium tuberculosis* Complex Isolates by PCR-Based Genomic Deletion Analysis - Parsons et al JCM 40:2339 -2345 (2002)

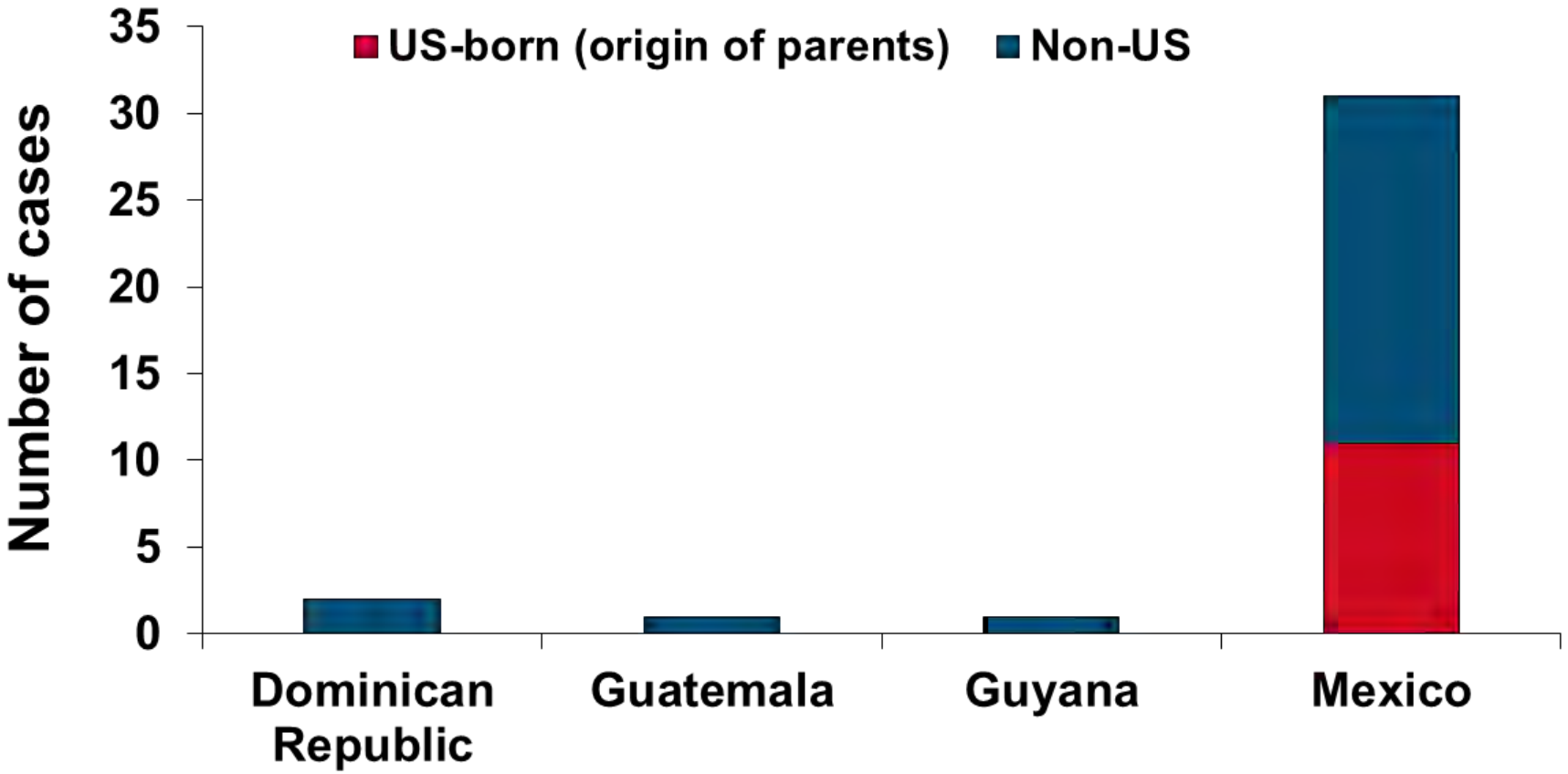
Human tuberculosis caused by *Mycobacterium bovis*

New York City

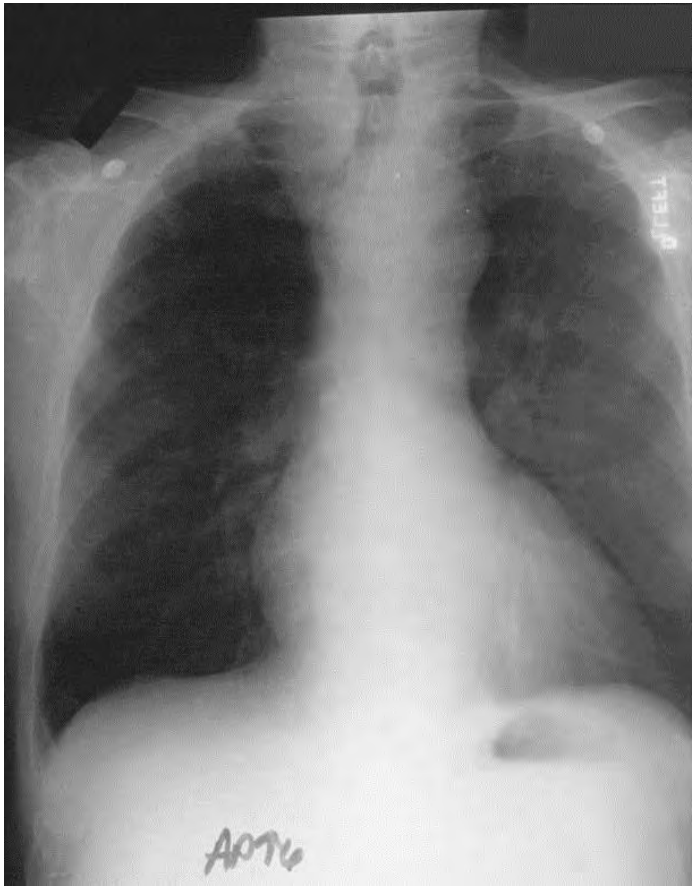
2001 - 2004

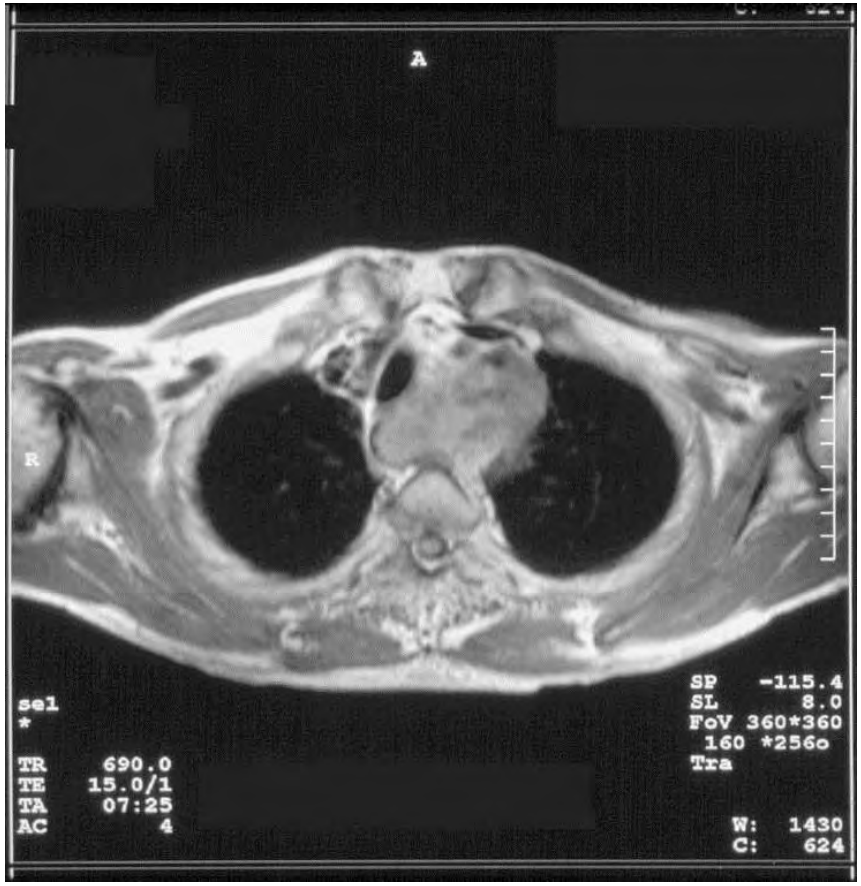
Winters et al 2005 MMWR 54:605-608

M. bovis cases, 2001-2004 (n=35)



78-year-old, bladder cancer - BCG





Somoskovi et al Eur J Clin Microbiol Infect Dis 26:937-940 (2007)

Identification

- ✓ *rpoB* gene or 16S rDNA sequencing, and *erm(*41*)/hsp65 analysis* for species differentiation within the *Mycobacterium abscessus* group

- ✓MALDI-TOF MS (Matrix-Assisted Laser Desorption ionization - time of flight mass spectrometry)

MALDI-TOF MS offers a rapid, protein-profiling based technique for identification of mycobacterial isolates from liquid or solid culture media, with high analytical capabilities at a less expensive cost compared to *rpoB* gene sequence analyses.

2. RIF resistance

yes

or

no?

Clinical course

Egg-based AST

Agar-based AST

Broth-based

***rpoB* analysis**

Mutation frequencies

- Within wild-type *M. tuberculosis* populations, small populations of mutants are found to be resistant to anti-TB drugs:

- **1.84×10^{-8} and 3.5×10^{-6} res to INH**
- **2.2×10^{-10} and 1.2×10^{-8} res to RIF**
- **1.0×10^{-7} and 3.1×10^{-5} res to EMB**
- **2.9×10^{-8} and 3.8×10^{-6} res to SM**

David H Applied Microbiology 1970 20:810-814

Do the math for MDR

- A mutant resistant to both INH and RIF would be really, really, really, really rare!
- **4×10^{-18} and 4.2×10^{-14}**

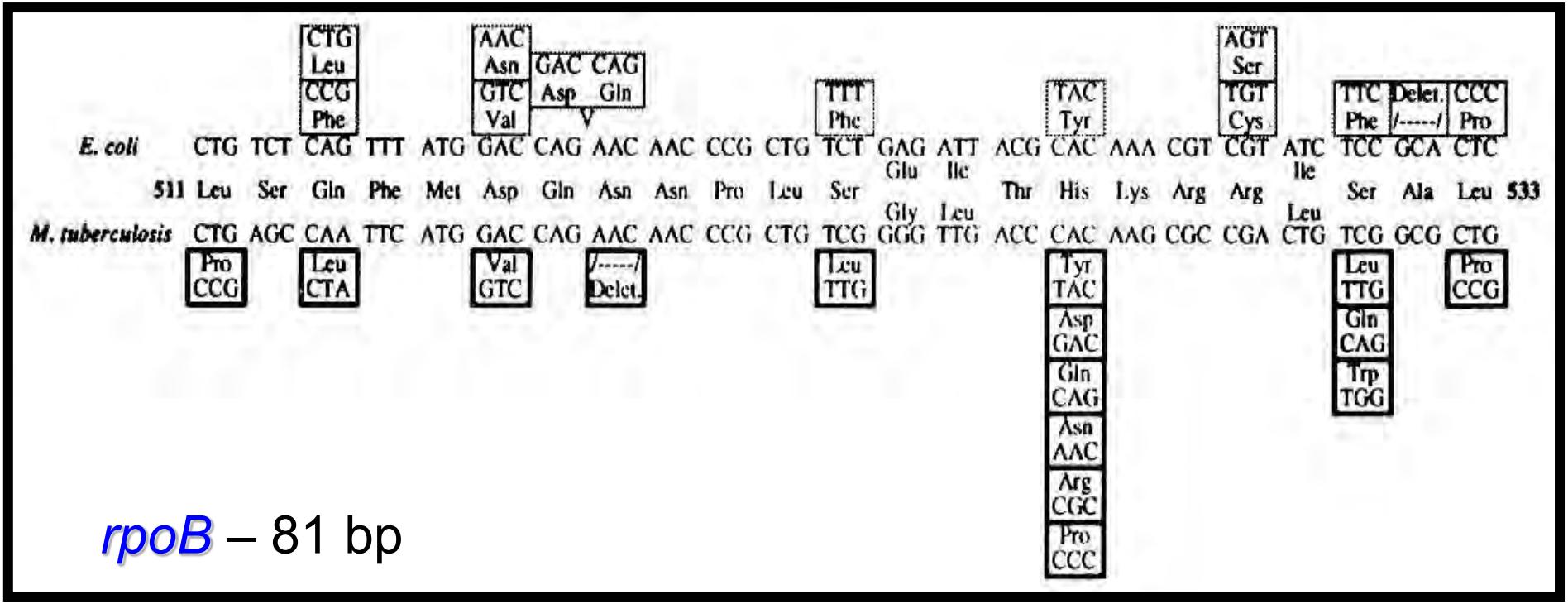
Do the math for MDR

- A mutant resistant to both INH and RIF would be really, really, really, really rare!
- **4×10^{-18} and 4.2×10^{-14}**

Canetti: a cavity lesion contains roughly **10^8** bacteria!

ARRD 1965 92:687-703

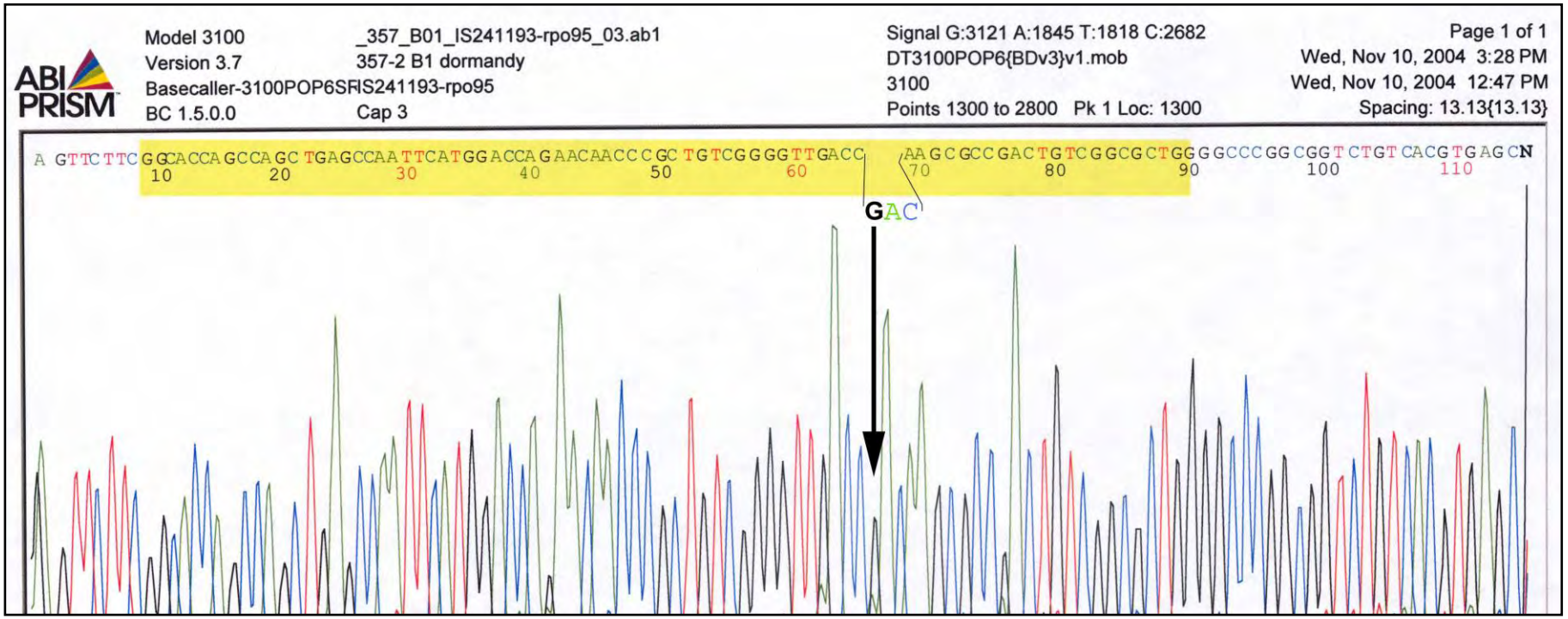
Detection of rifampin-resistant mutations in *M. tuberculosis*



rpoB – 81 bp

Lancet. 1993 Mar 13;341(8846):647-50. Detection of rif-resistance mutations in *M. tuberculosis*. Telenti A et al.

rpoB analysis



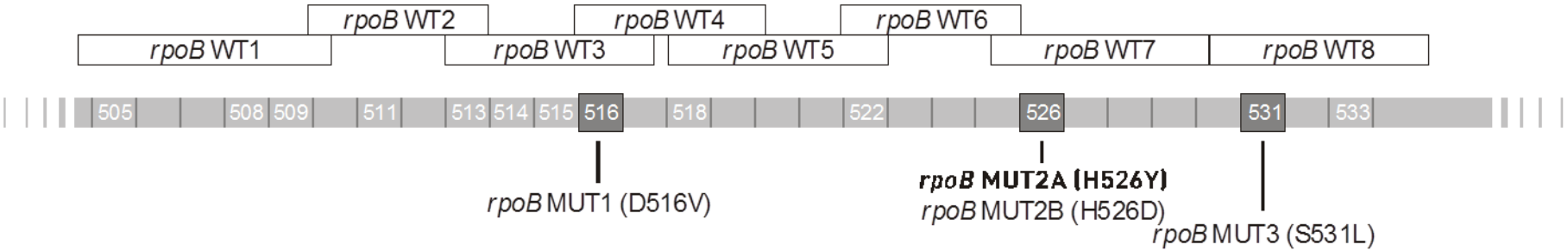
Codon 526 (Cytosine-Adenine-Cytosine) encodes histidine in a susceptible strain; replaced with (Guanine-Adenine-Cytosine) aspartate in a resistant strain.

It's easier now…

- **INNO-LiPA Rif. TB***
  - Innogenetics, Belgium
- **GenoType MTBDR*plus****
  - Hain Lifescience, Germany
- **GeneXpert MTB/RIF***
  - Cepheid, California
- **NTM+MDRTB Detection Kit 2**
  - Nipro Corporation, Japan

*These assays endorsed by WHO (2008)

RIF Resistance Region – *rpoB* Gene

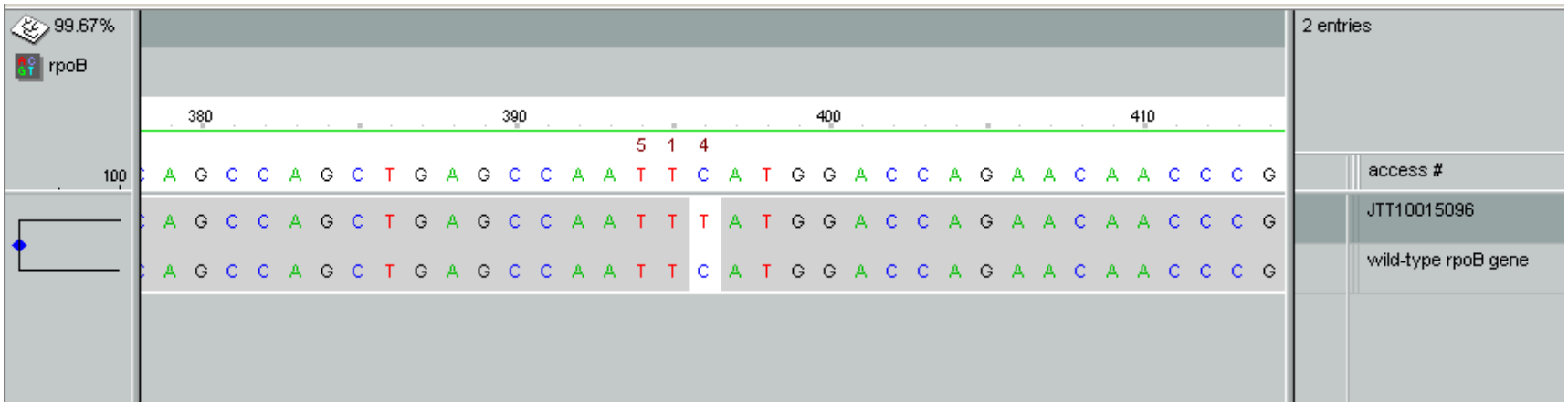


rpoB-Wildtype-probes: WT 1 to WT 8

rpoB-Mutation-probes: MUT D516V, H526Y, H526D, S531L

Detection of mutations through missing of wildtype signals

No mutant band visible



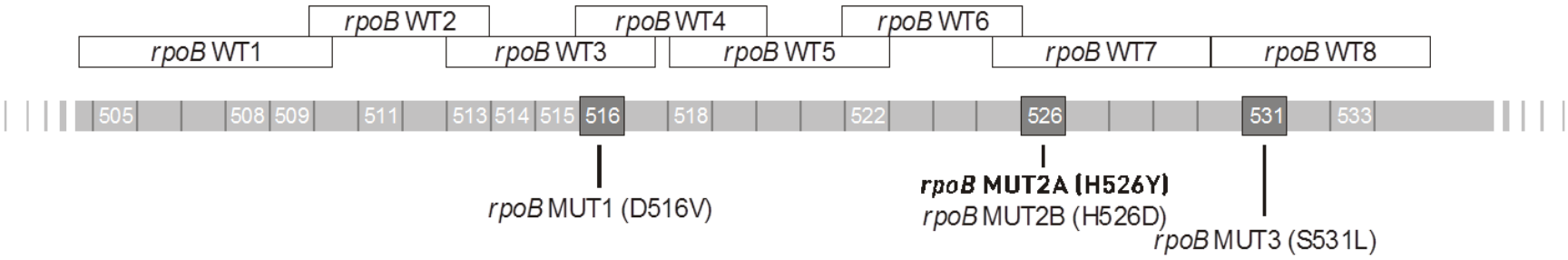
At position 514:

Codon TTC encodes for Phenylalanine - wild-type

Codon TTT encodes for Phenylalanine - JTT10015096

Silent mutation: Nucleotide changes that do not result in a change in amino acid sequence

RIF Resistance Region – *rpoB* Gene



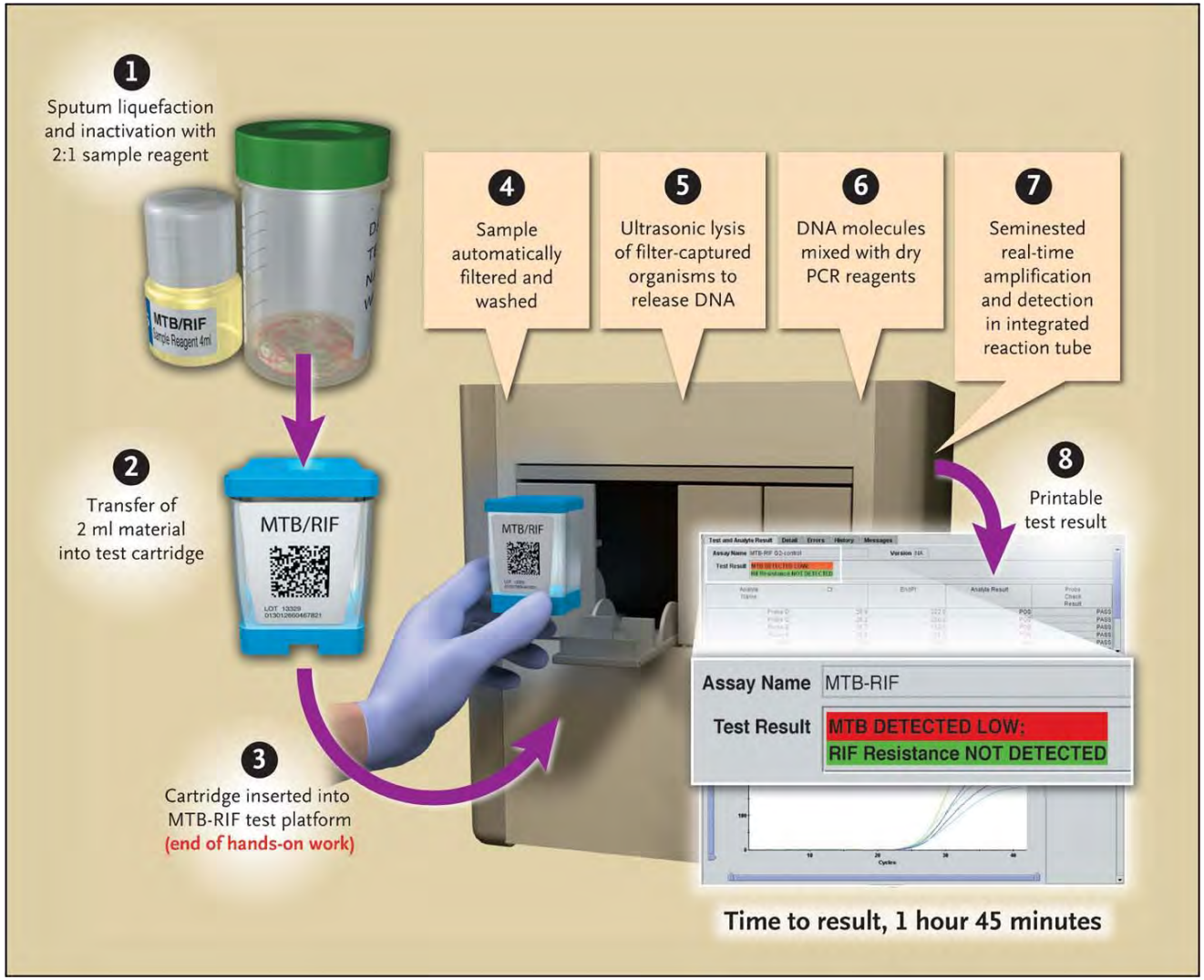
rpoB-Wildtype-probes: WT 1 to WT 8

rpoB-Mutation-probes: MUT D516V, H526Y, H526D, S531L

Detection of mutations through missing of wildtype signals

Detection of mutations through presence of mutation signals

MTB/RIF Test



Boehme CC et al. N Engl J Med 2010;363:1005-1015

rpoB – 20 years later

- *In July 2013, the FDA granted Market Clearance to the Cepheid Xpert® MTB/RIF assay. This NAA test can simultaneously identify* Mycobacterium tuberculosis *complex (MTBC) and genetic mutations associated with resistance to rifampin from raw sputum and concentrated sputum sediments.*
- *The assay utilizes a self-contained, disposable cartridge that can be used on Cepheid's fully-automated GeneXpert® Instrument Systems. The convenience and automation of this system has the potential to provide rapid access to patient results.*

APHL

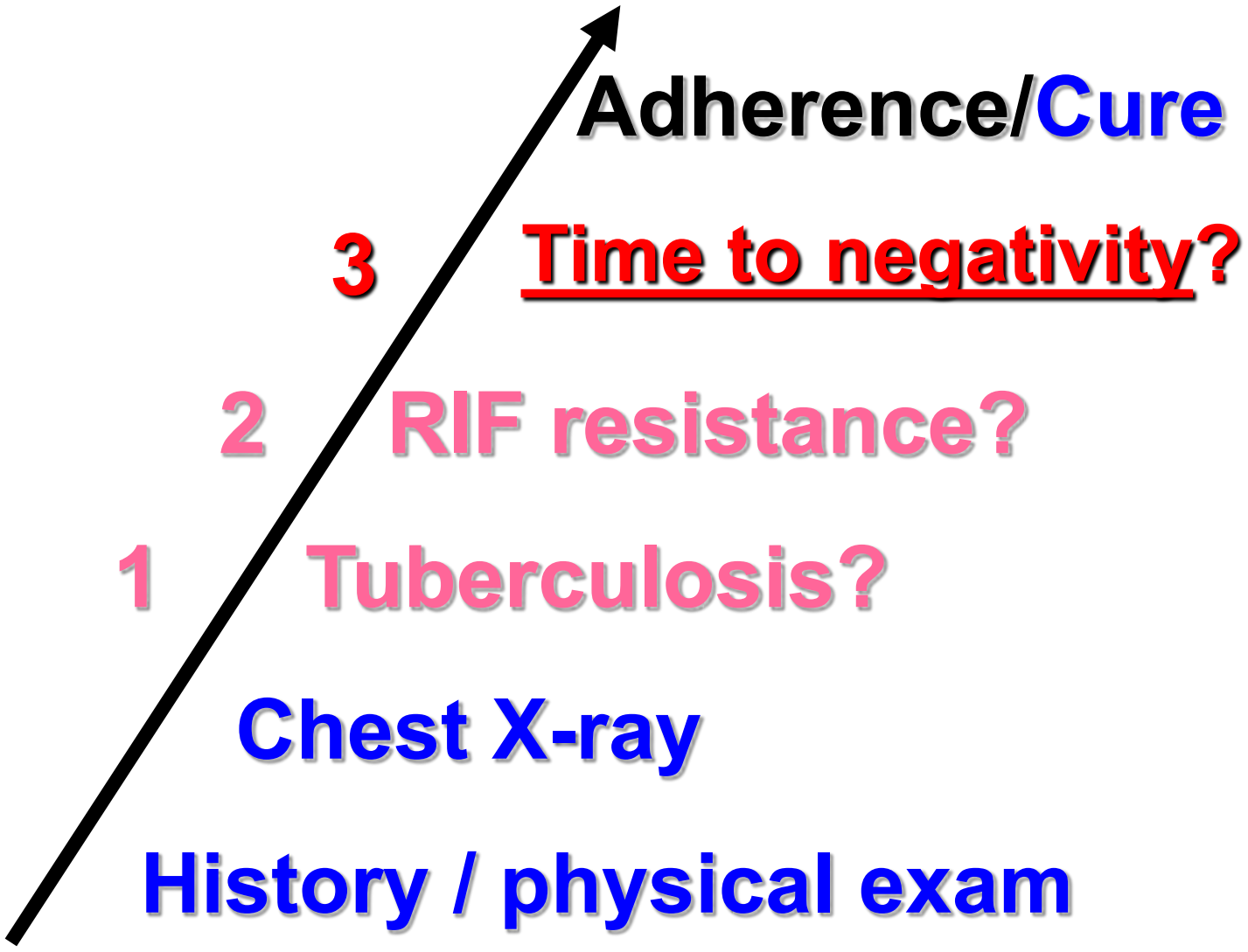
Xpert® MTB/RIF only performed

… the following actions must be taken:

- **It is strongly recommended that specimen be sent to a reference laboratory for AFB smear and culture as soon as possible regardless of the NAA result.** If there is a sufficient volume of raw sputum, split the specimen and send to a reference laboratory for both concentrated AFB smear and culture. The sample must be split prior to the laboratory mixing a sputum sample with the Sample Reagent (SR). If volume is insufficient, request an additional sputum specimen for AFB smear and culture.
- Report results from the Xpert® MTB/RIF assay as soon as available while awaiting culture confirmation.
- **If RIF resistance is detected, a specimen should be sent to a reference laboratory to confirm the resistance by DNA sequencing as soon as possible.**

APHL Factsheet September 2013

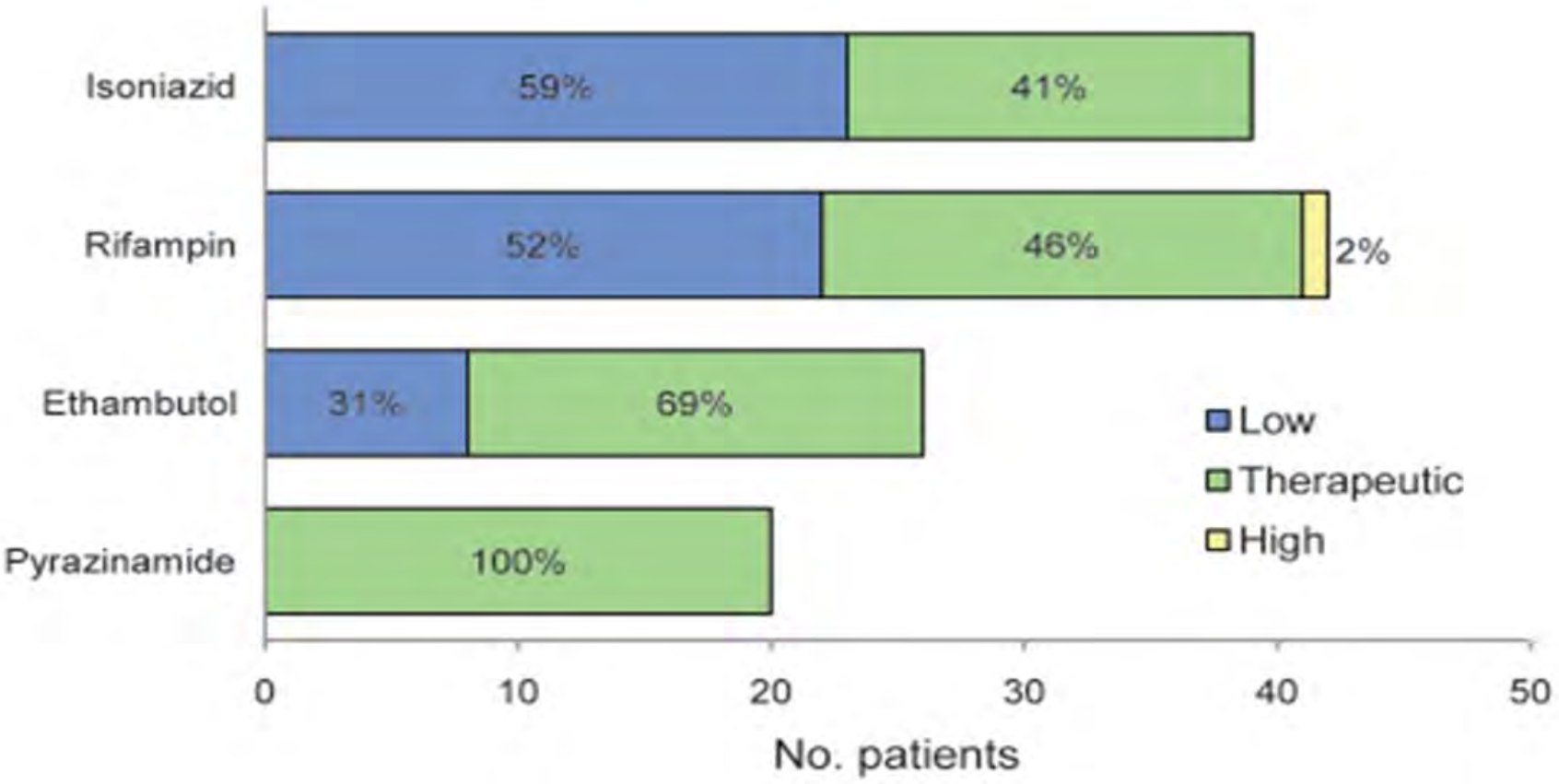
ATS/CDC/IDSA Treatment Guidelines 2003



Follow up specimens…

- 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

Therapeutic drug monitoring

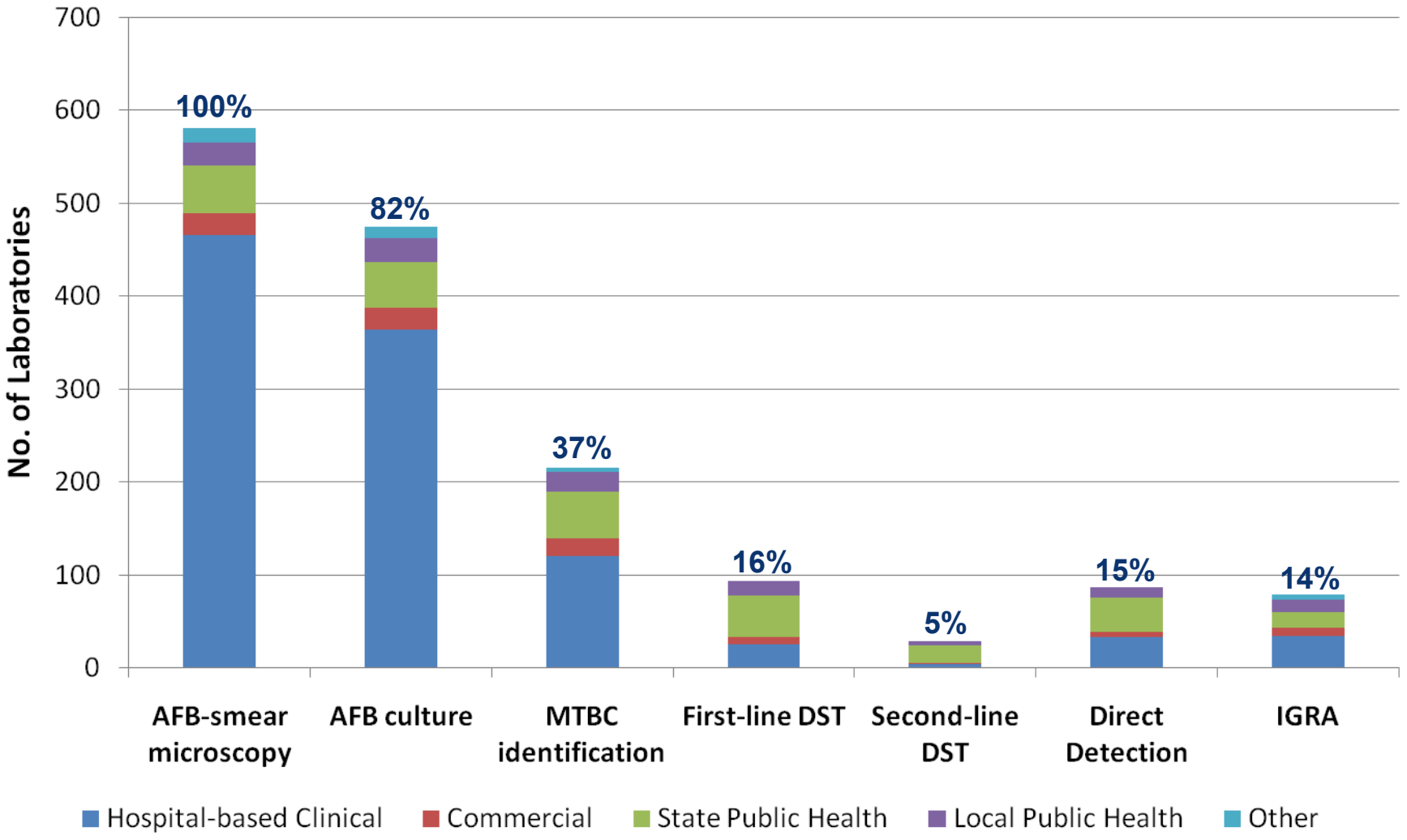


Heysell et al. *Therapeutic Drug Monitoring for Slow Response to Tuberculosis Treatment in a State Control Program, Virginia, USA*
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 10, October 2010

Topics

- Introduction
- Epidemiology – USA and global
- 3 important questions
- **Systems thinking**

In-house service performed- APHL/CDC Survey 2011



ThermoFisher SCIENTIFIC

Florida Dept. of Health receives 2012 ASTHO Vision Award

ThermoFisher SCIENTIFIC

Quality testing requires quality specimen

5 to 10 ml sputum

Toolbox-1

✓**Specimen – sputum, CSF, formalin-fixed tissue**

- AFB microscopy
- Solid & broth-based media
- **NAAT-D** (TB complex)
- **NAAT-R** (RIF, INH and more)
- **Direct AST**

✓**Patient management** (culture negativity after 2 months on treatment)

Molecular TB Testing 7 Days a Week

Toolbox-2

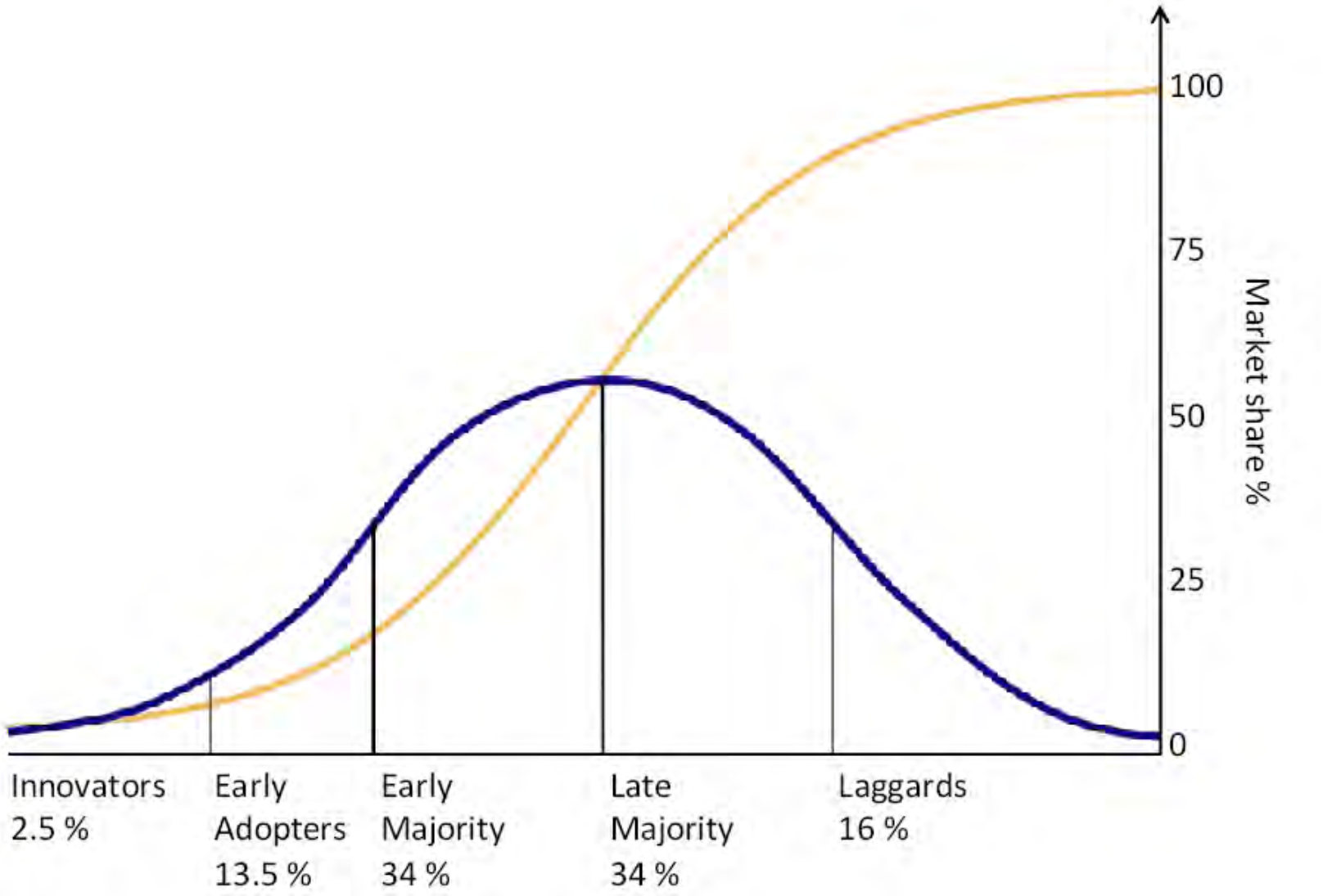
- ✓**AFB positive culture (broth-, solid-based media)**
  - **TB Yes/No** (final identification within TB complex)
  - **NAAT-R**
  - Broth-based AST
  - Agar-based AST
  - Minimum Inhibitory Concentration (MIC)
- ✓**Population management/genotyping**
  - RFLP-IS*6110*, Spoligo and MIRU
  - Whole genome analysis on the horizon
  - Standardization through contracted PHL-MI

Diffusion of Innovations- 1958

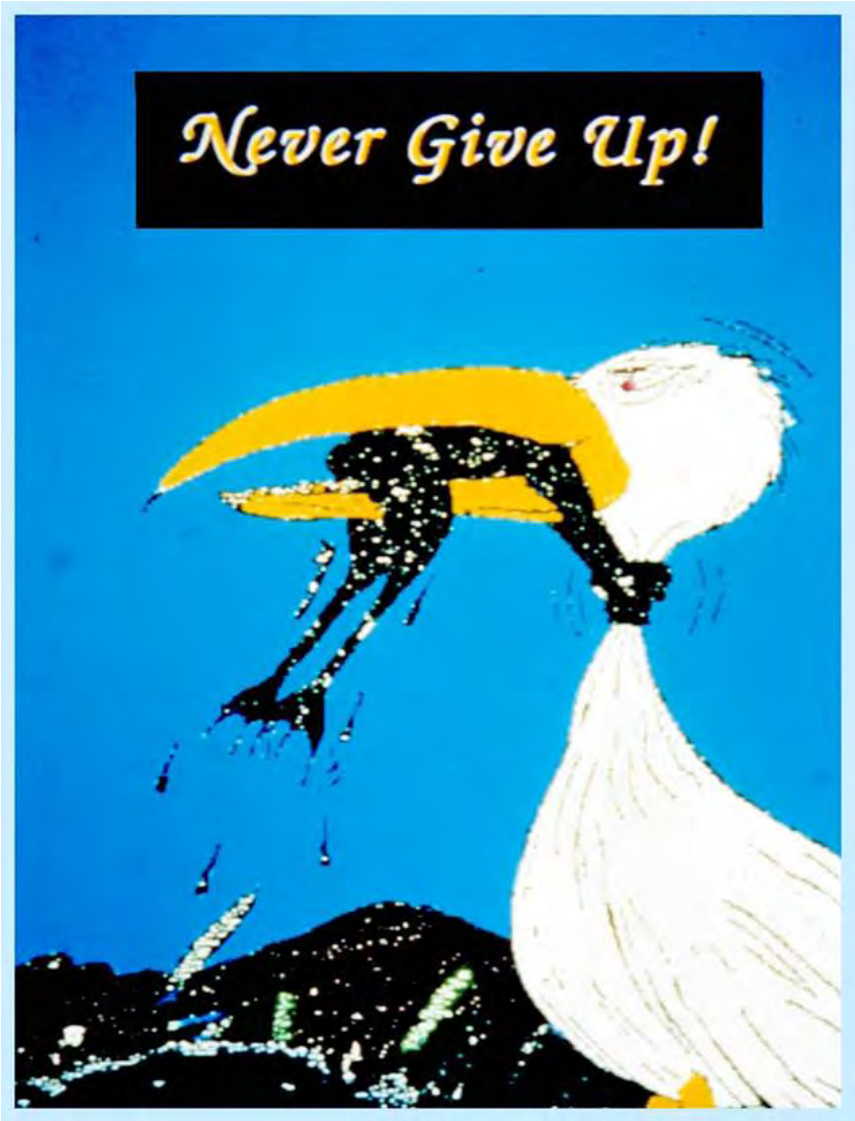
- Innovators 2.5%
- Early Adopters 13.5%
- Early Majority 34%
- Late Majority 34%
- Laggards 16%

Everett M. Rogers, 1931-2004

Diffusion of innovations



Rogers –adapted by Tungsten



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Thank you!

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Questions?

Max Salfinger, M.D.

National Jewish Health – Denver

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For participation in the December 10, 2015 webinar titled

The Race is On: Faster Turnaround Times in the Diagnosis of Multidrug-Resistant Tuberculosis

Webinar Presenter:

Dr. Max Salfinger

National Jewish Health, Denver, CO

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