UNIVERSITY OF SOUTH FLORIDA

A PREEMINENT RESEARCH UNIVERSITY

What are the Current Roles of AFB Smear Microscopy and TB NAAT in the Diagnosis and Management of Mycobacterial Diseases?

Max Salfinger
University of South Florida
College of Public Health & Morsani College of Medicine
Tampa, Florida
September 13, 2022
Portsmouth, NH

From Albany to Bismarck…

Declaration

Travel support from
MetaSystems
&
I enjoy contributing towards **a world free of TB**

Learning objectives

As a result of attending this session, participants will be able to:

- **Describe improvements for the acid-fast bacilli stain.**
- **Explain the difference of the rule-in versus rule-out indication of TB testing.**
- **Discuss Roger's diffusion of innovation theory and how it relates to clinical microbiology.**

Laboratory's charge

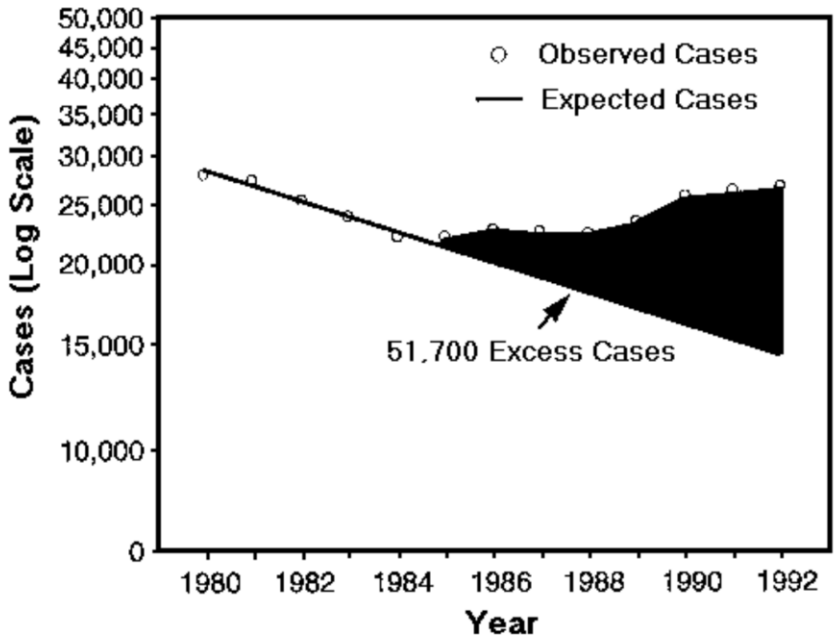
To provide the healthcare provider with accurate results in a timely fashion

Topics

- **State of the TB epidemic**
- Quantum leaps in mycobacteriology during last 50 years
- AFB stain - still relevant?
- TB NAAT – still a frontier?
- Systems
- Closing remarks

The unexpected happened

FIGURE 1. Expected and observed number of tuberculosis cases – United States, 1980-1992



1992 & 2021 TB cases & incidence

	1992		2021		
CT	156	4.8	54	1.5	
ME	24	1.9	14	1.0	
MA	428	7.1	151	2.2	
NH	18	1.6	12	0.9	
NY	4,574	25.2	681	3.4	
RI	54	5.4	17	1.6	
VT	7	1.2	2	0.3	
NACMID	5,261		931		5.7
USA	26,673	10.4	7,860	2.4	3.4

Congratulations – NACMID states

Constitution State

Vacationland

The Sprit of America

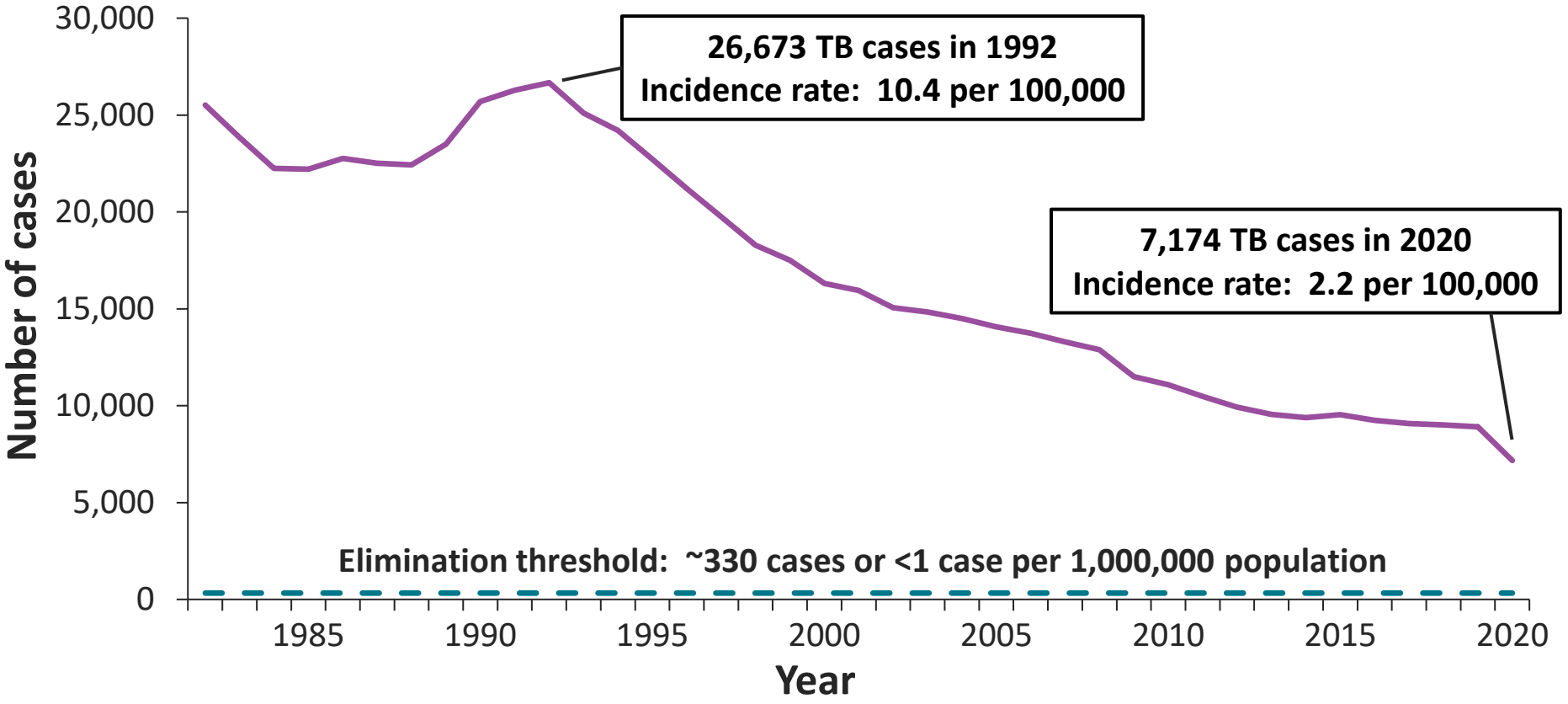
Live Free or Die

Excelsior

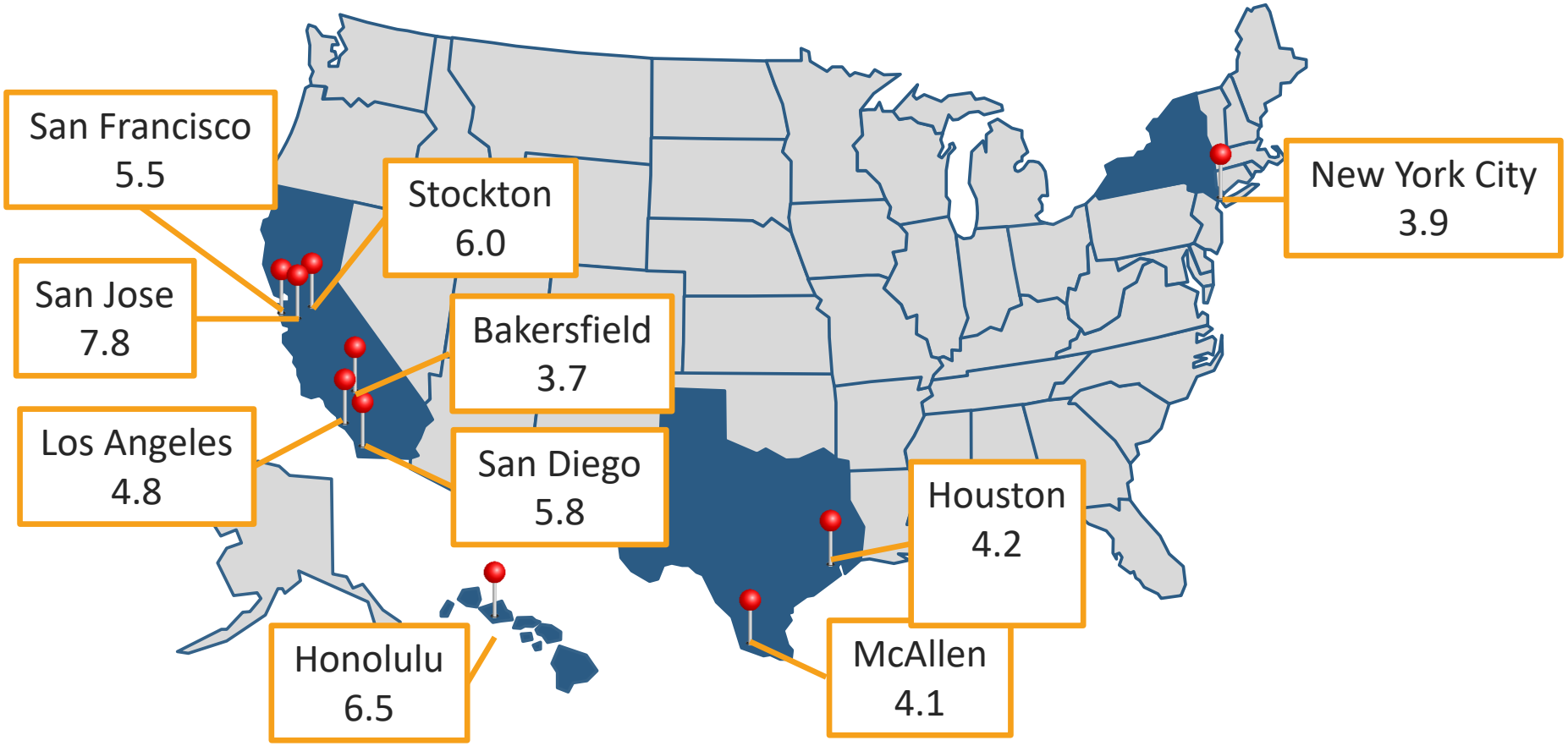
Ocean State

Green Mountain State

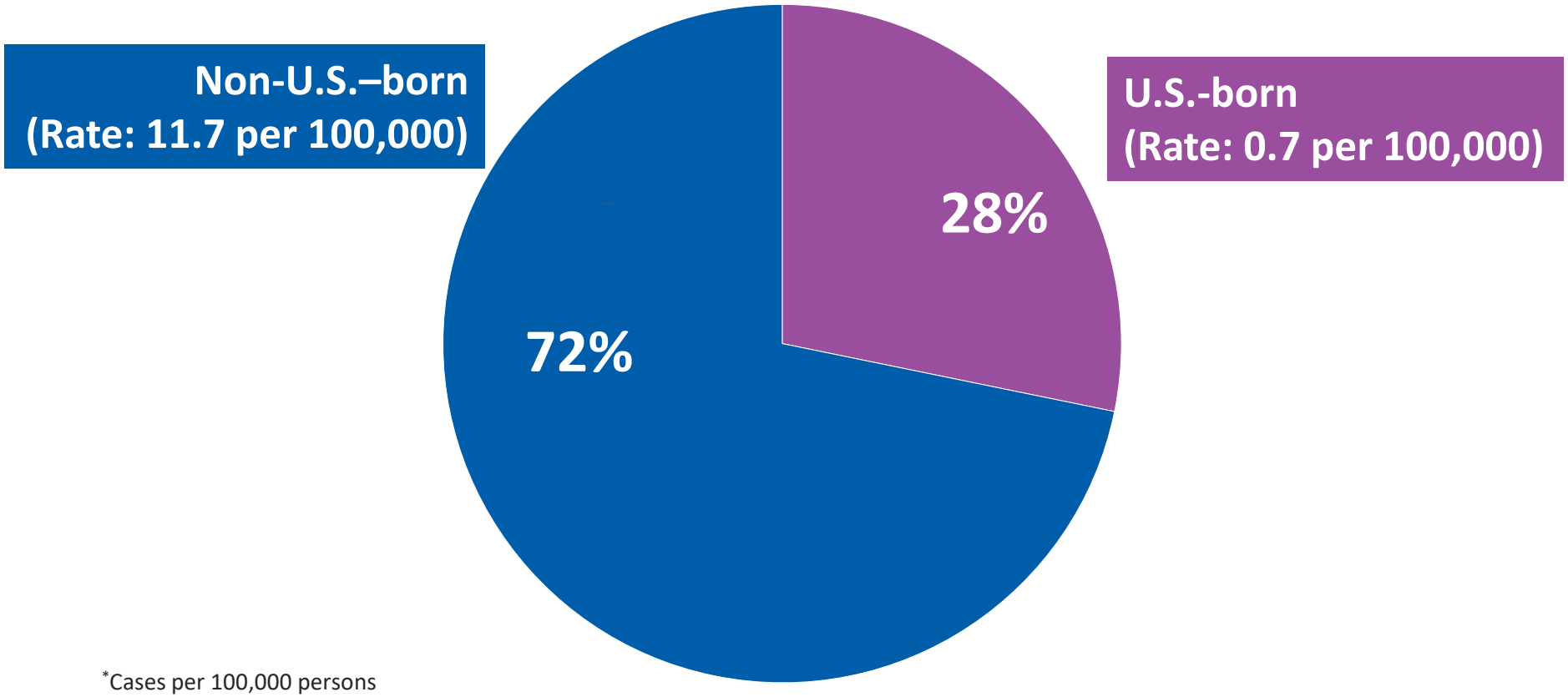
Progress towards TB elimination, United States, 1982–2020



Top 10 TB incidence rates by Metropolitan Statistical Areas (MSAs), United States, 2020

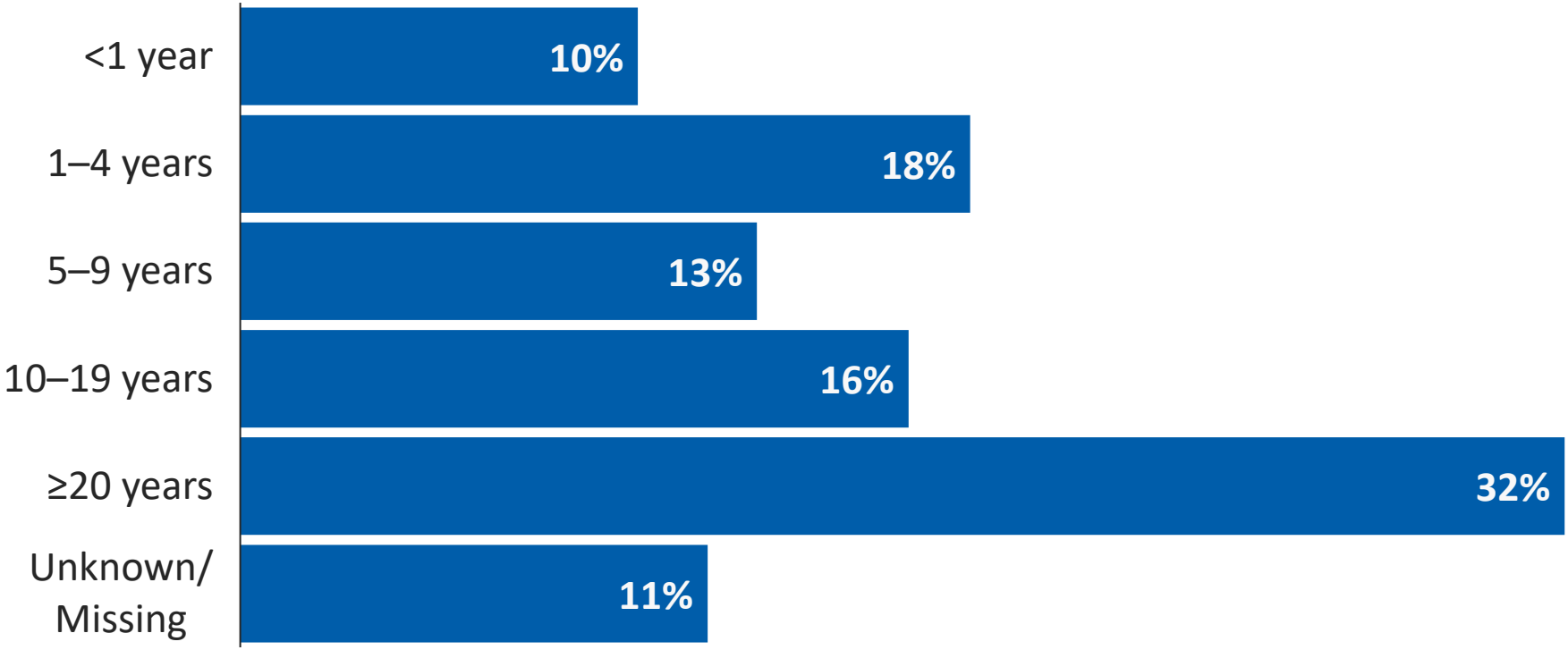


TB incidence rates* and percentages by origin of birth, United States, 2020 (N=7,145)

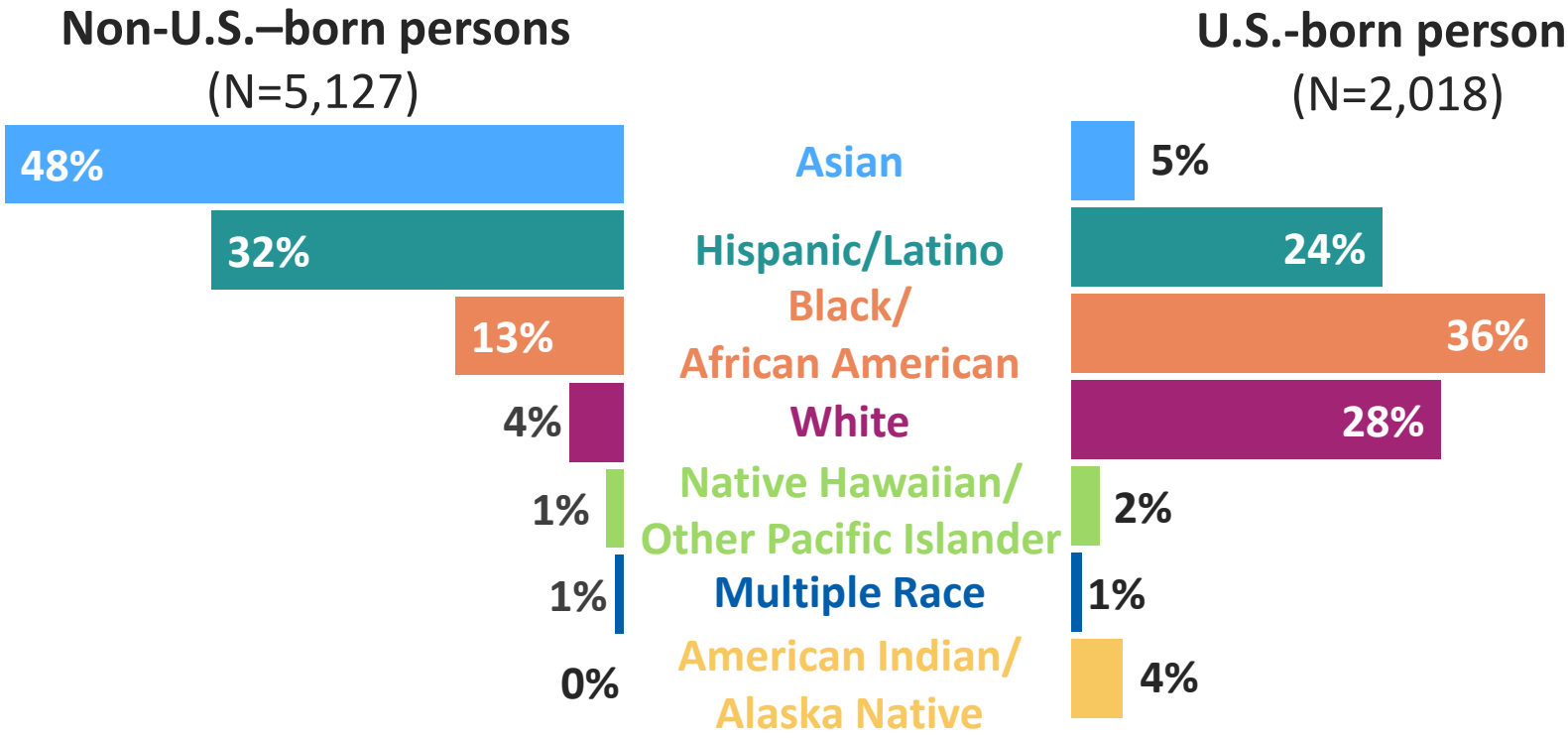


*Cases per 100,000 persons

Percentage of TB cases among non-U.S.–born persons by years since initial arrival in the United States at diagnosis, 2020 (N=5,127)



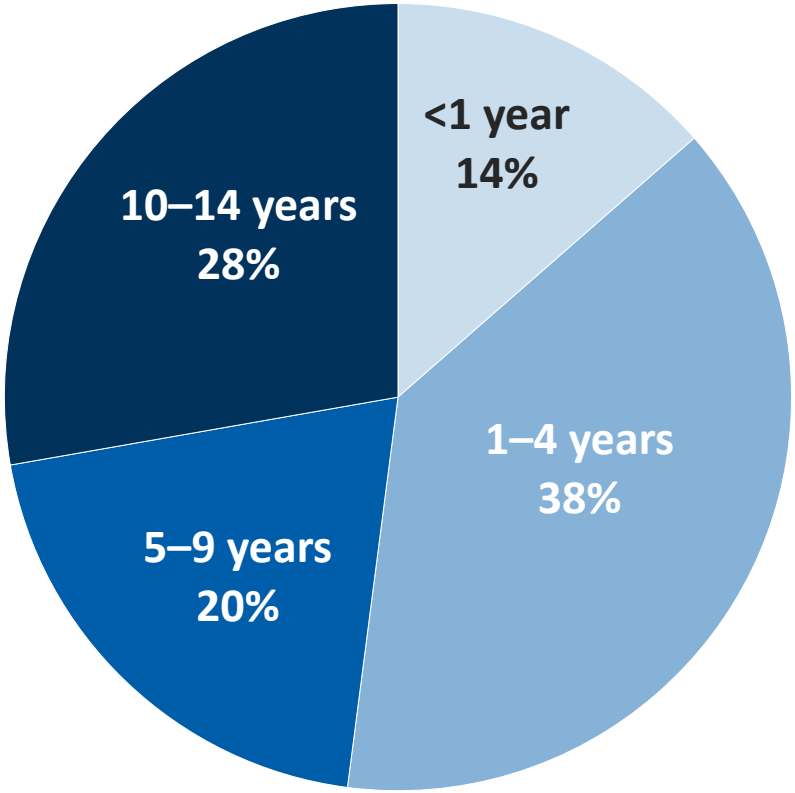
Percentage of TB cases by origin and race/ethnicity,* United States, 2020†



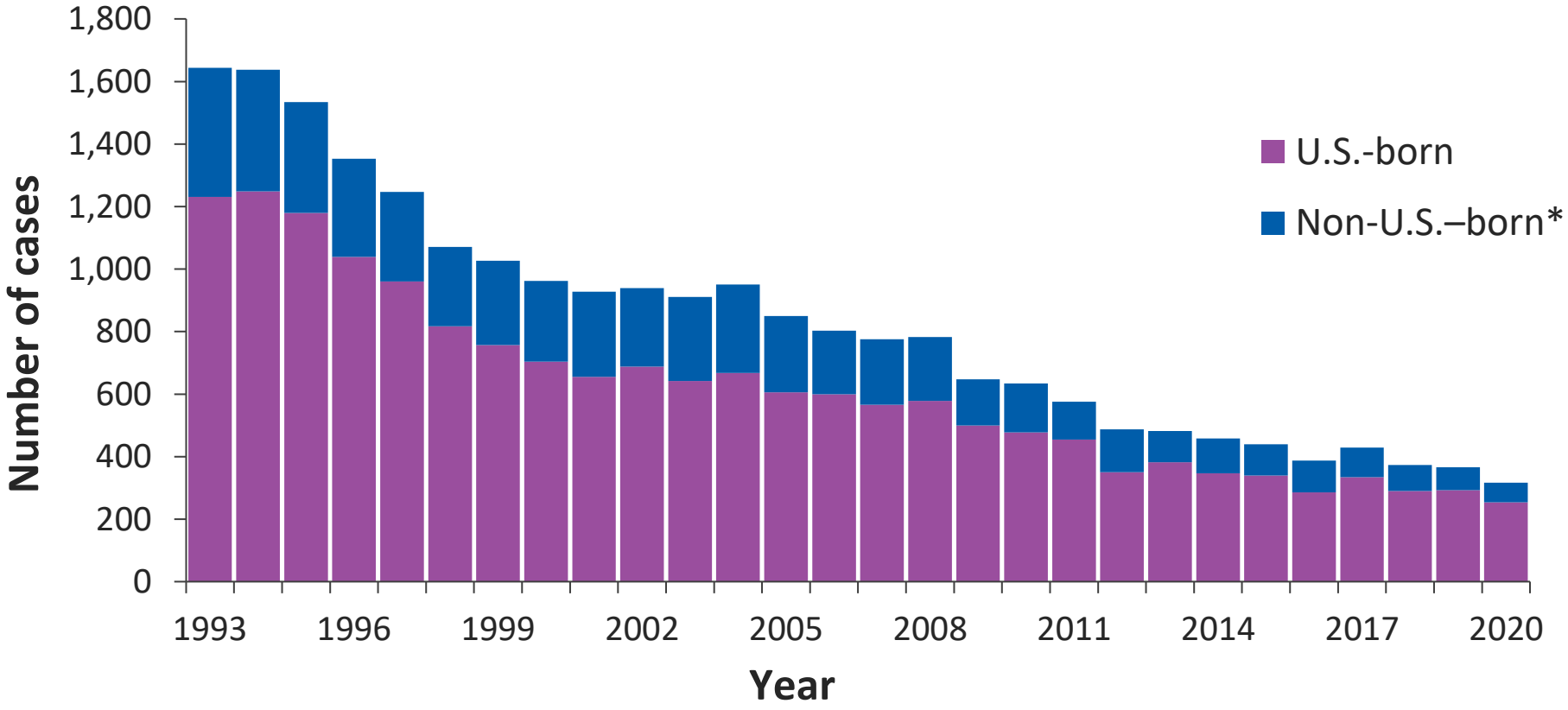
* All races are non-Hispanic; multiple race indicates two or more races reported for a person but does not include persons of Hispanic or Latino origin.

† Percentages are rounded. Percentages of unknowns/missing are <1% and are not displayed in graphs.

Percentage of pediatric TB cases by age group, United States, 2020 (N=317)

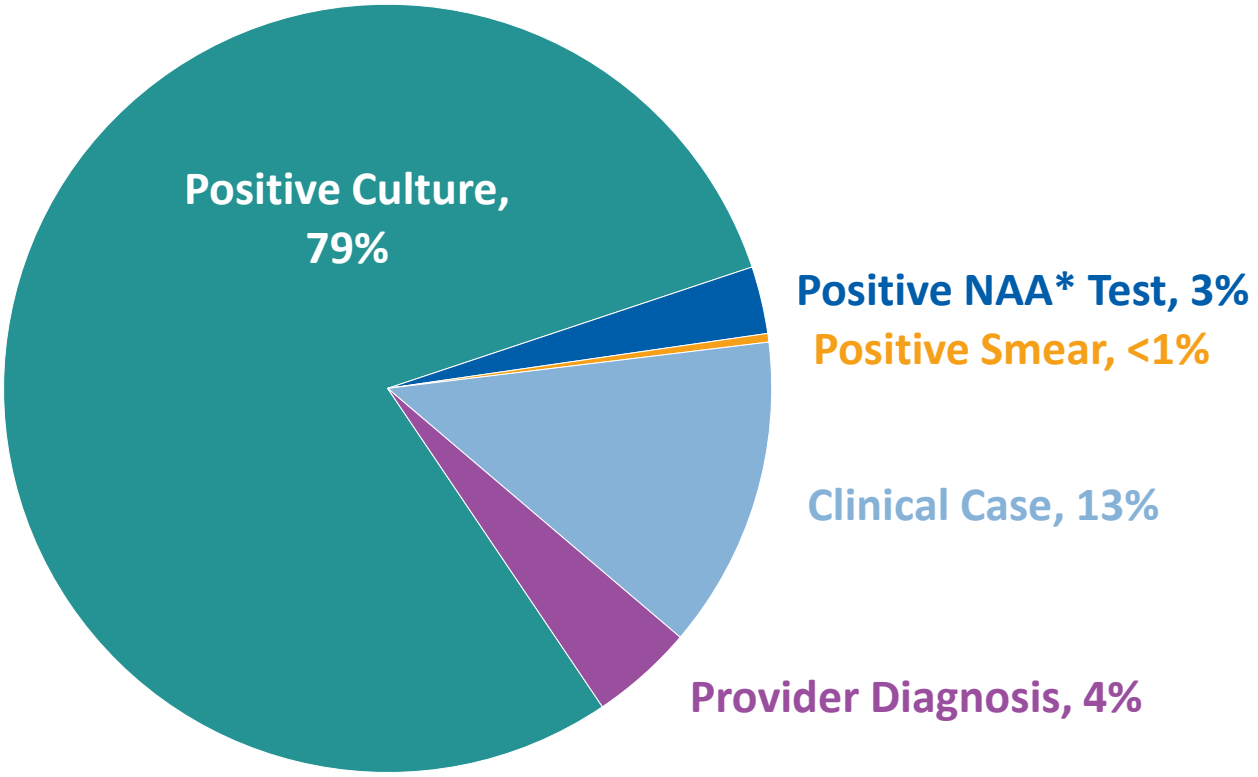


Pediatric TB cases by origin of birth, United States, 1993–2020



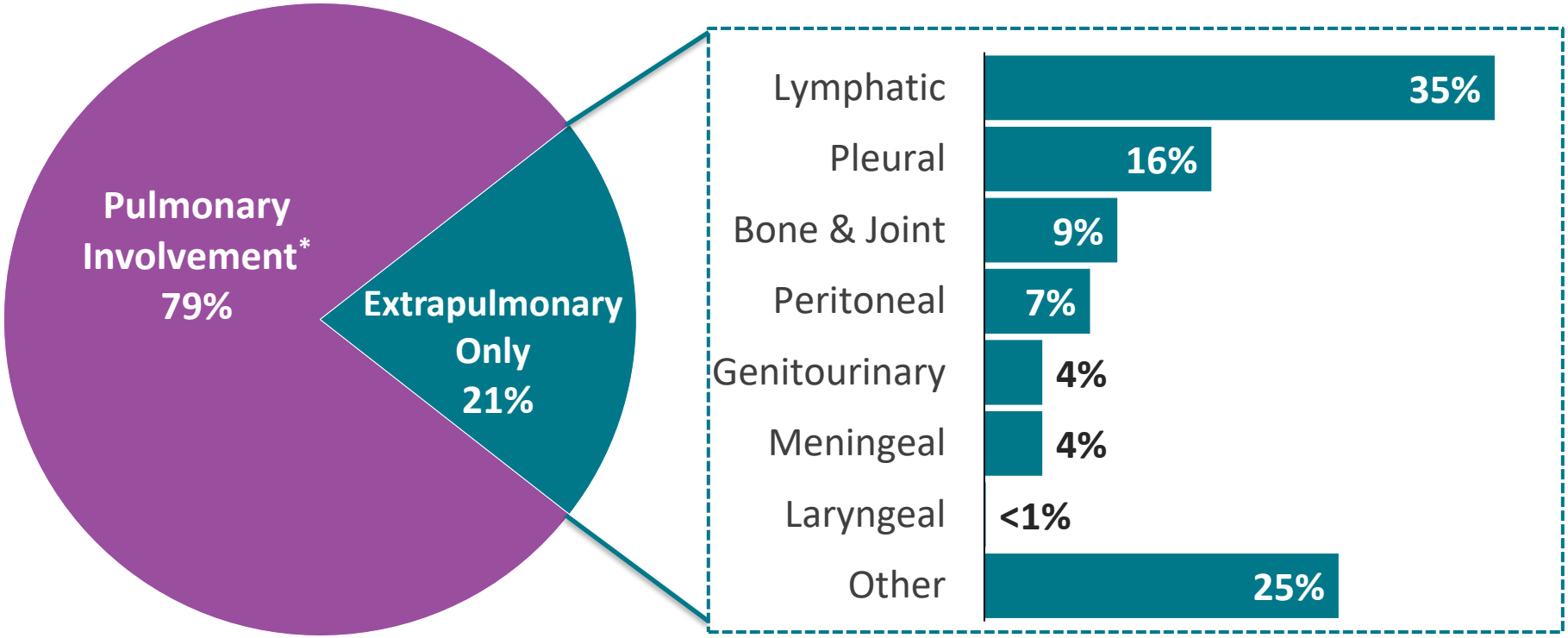
*Non-U.S.–born refers to persons born outside the United States or its territories or not born to a U.S. citizen

Percentage TB cases by case verification criteria, United States, 2020 (N=7,174)



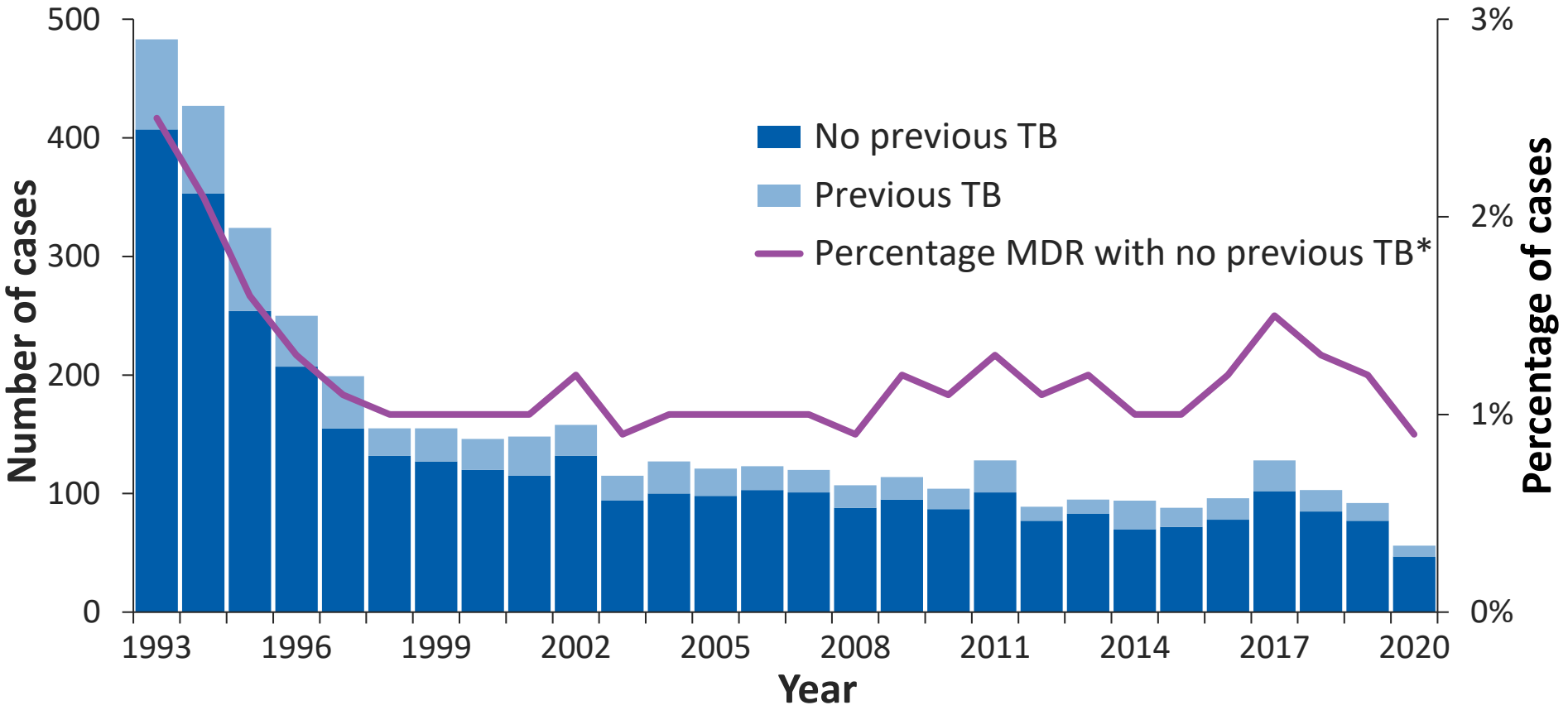
*NAA=nucleic acid amplification

Percentage of TB cases by site of disease, United States, 2020



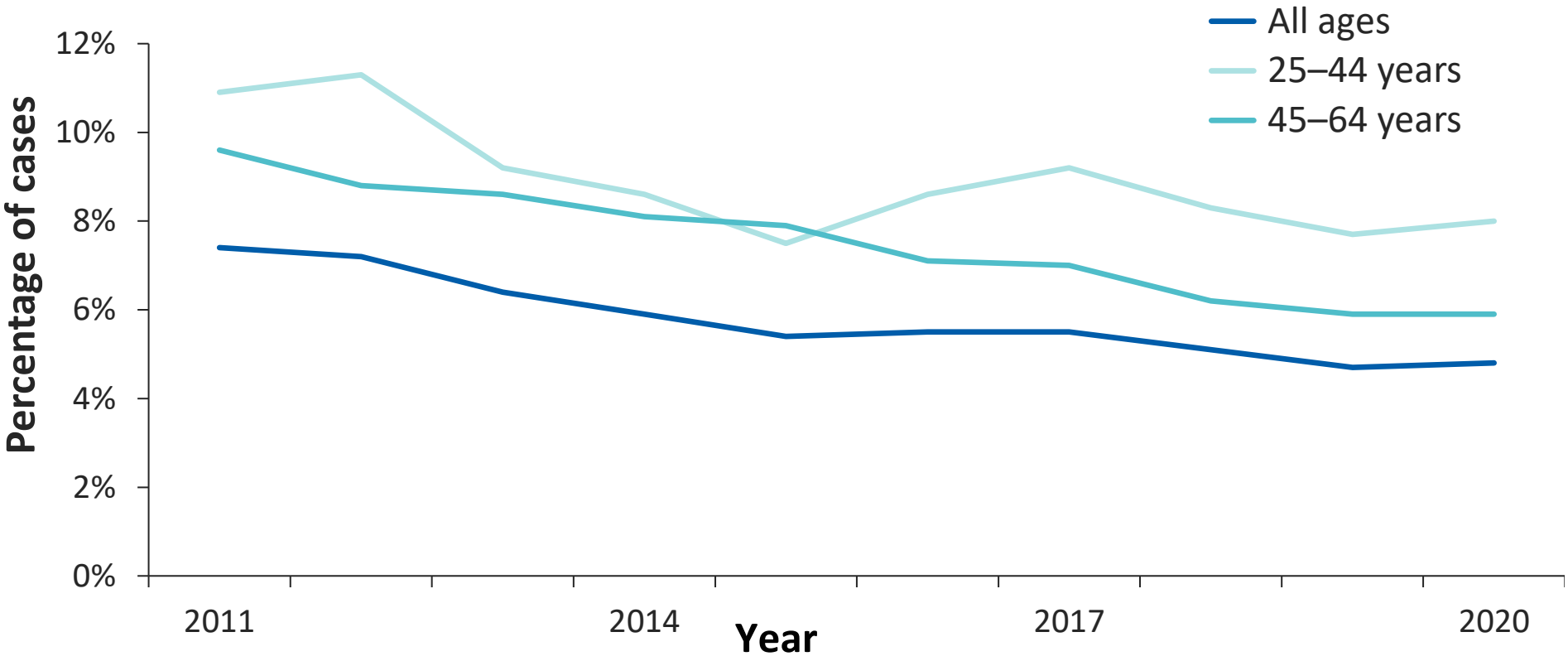
*Any pulmonary involvement which includes cases that are pulmonary only and both pulmonary and extrapulmonary.
Patients may have more than one disease site but are counted in mutually exclusive categories for surveillance purposes.

Cases and percentages of MDR TB by history of TB, United States, 1993–2020

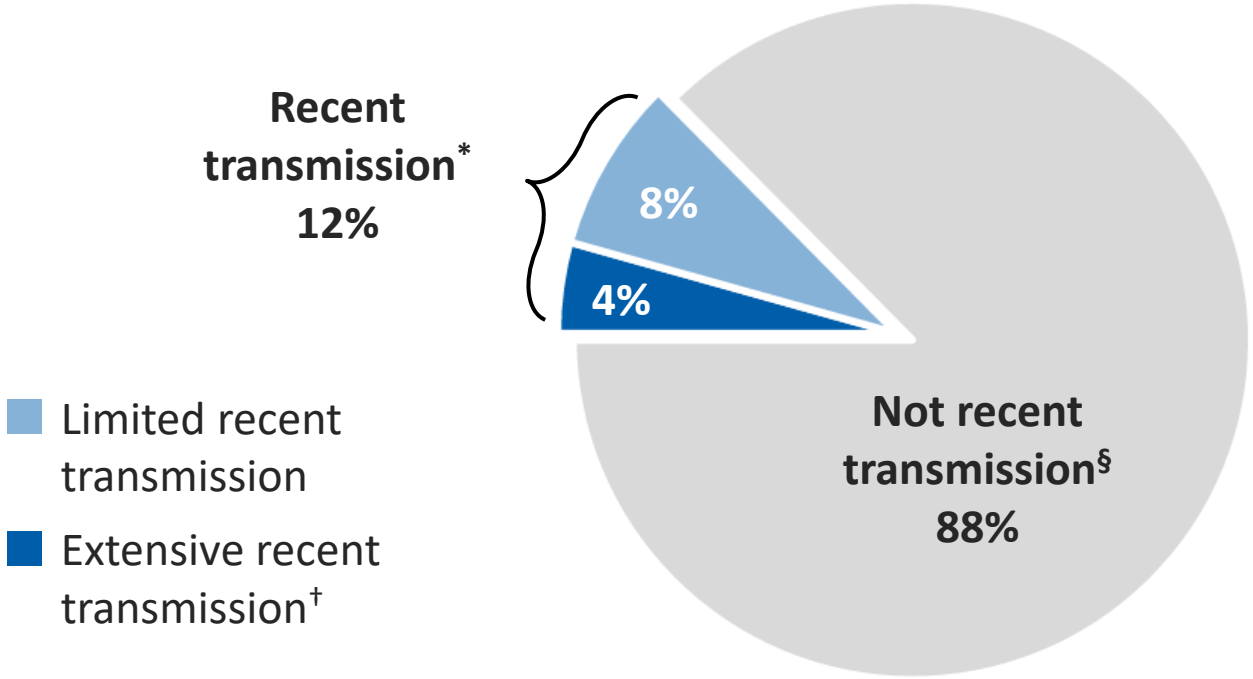


*Based on initial isolates from persons with no prior history of TB; multidrug-resistant TB (MDR TB) is defined as resistance to at least isoniazid and rifampin.

Percentage of HIV coinfection by age among persons with TB, United States, 2011–2020



Genotyped TB cases estimated to be attributed to recent transmission, United States, 2019–2020 (N=12,242)



* A TB case is designated as attributed to recent transmission if a plausible source case can be identified in a person who i) has the same *M. tuberculosis* genotype, ii) has an infectious form of TB disease, iii) resides within 10 miles of the TB case, iv) is 10 years of age or older, and v) was diagnosed within 2 years before the TB case.

† A TB case is designated as attributed to extensive recent transmission when the criteria above for recent transmission are met, and furthermore the case belongs to a plausible transmission chain of six or more cases. Otherwise, the case is designated as attributed to limited recent transmission.

§ Cases not attributed to recent transmission may be misclassified in children <5 years old or indeterminate in persons with a recent U.S. arrival due to limitations of the plausible-source case method.

California – AFB smear Pulmonary cases only

Sputum Smear Status of Pulmonary Cases	2016 (No.)	2016 (%)	2017 (No.)	2017 (%)	2018 (No.)	2018 (%)	2019 (No.)	2019 (%)	2020 (No.)	2020 (%)
All Pulmonary Cases	1699	100.0	1669	100.0	1722	100.0	1739	100.0	1365	100.0
Positive	856	50.4	837	50.1	818	47.5	865	49.7	669	49.0
Negative	757	44.6	740	44.3	814	47.3	780	44.9	605	44.3
Not Done	86	5.1	90	5.4	89	5.2	94	5.4	90	6.6
Unknown	.	.	2	0.1	1	0.1	.	.	1	0.1

Topics

- State of the TB epidemic
- **Quantum leaps in mycobacteriology during last 50 years**
- AFB stain - still relevant?
- TB NAAT – still a frontier?
- Systems
- Closing remarks

New assays/techniques

1977 Middlebrook G – Radiometric broth medium
1983 Roberts GD – Radiometric antimicrobial susceptibility testing
1987 Roberts MC – DNA probes for identification
1991 Cave MD – IS*6110* for fingerprinting
1991 Eisenach KD – PCR from sputum
1992 Boettger EC – *Mycobacterium genavense* (sequencing)
1993 Telenti A – *rpoB* sequencing (rifampin resistance marker)
1993 Jonas V - Amplified Mycobacterium Tuberculosis Direct Test
1999 Hanna BA – Walk away system for growth detection
2006 Somoskovi A – MDR-TB screen in AFB+ sputum (LPA)
2006 Richter E – DNA strip assay for identification of mycobacteria
2010 Helb D – Fully integrated sample processing (cartridge-based)
2011 Saleeb PG – MALDI-TOF MS for identification of mycobacteria
2016 Pankhurst LJ – Whole genome sequencing (WGS) for TB & antimicrobial resistance markers

Topics

- State of the TB epidemic
- Quantum leaps in mycobacteriology during last 50 years
- **AFB stain - still relevant?**
- TB NAAT – still a frontier?
- Systems
- Closing remarks

History of AFB ZN stain

1882 - **Koch** used hot alkaline methylene blue as the primary stain and vesuvin as decolorizer and counterstain.

1882 – **Ehrlich** used fuchsin as the primary stain, aniline as the mordant, and a mineral acid as the decolorizer.

1882 – **Ziehl** used phenol as the mordant

1882 – **Rindfleisch** heated the slide instead of putting into hot water

1883 – **Neelsen** combined Ehrlich's fuchsin with Ziehl's mordant

Ziehl-Neelsen stain as we know it today should be properly called:

Koch-Ehrlich-Ziehl-Rindfleisch-Neelsen stain

References: Bishop & Neumann (Tubercle 1970); Allen & Hinkes (Bull Int Union Tuberc 1982)

Ziehl-Neelsen stain

Preparation

1. Add 100ml of ethanol (or methanol) to a one litre glass flask
2. Add 50g of phenol crystals and dissolve
3. Add 10g of basic fuchsin powder
4. Mix well until dissolved
5. Add distilled water to make one litre
6. Label the bottle – **"1% carbol fuchsin", date and initial**
8. Store in a dark bottle in a cupboard at room temperature (expiry 12 months)

https://stoptb.org/wg/gli/assets/documents/TBLabDiagnosisSputum%20Microscopy_Handbook.pdf Accessed: September 1, 2022

Direct versus concentrated smear

ZN **Direct: 70.5%** **Concentrated: 82.9%**

BMC Res Notes. 2013; 6: 291

AR/AO **Direct: 81%** **Concentrated: 91%** (3 sputum samples)

J Clin Microbiol. 1999 Nov; 37(11): 3564–3568

ZN **Direct: 51%** **Concentrated: 52%**

(first early morning sputum from HIV-positive patients suspected of pulmonary TB)

BMC Infectious Diseases volume 9, Article number: 53 (2009)

Carbol fuchsin versus fluorescence

In 2011, WHO released a new policy on Light Emitting Diode (LED) based Fluorescent Microscopy (FM) for diagnosing TB. FM is equally accurate, at least 10% more sensitive and has qualitative, operational, cost and workload advantages for all laboratories performing sputum smear microscopy. WHO recommended a phased approach to change from brightfield microscopy to LED-based FM across the microscopy network.

LED FM offers considerable advantages over conventional FM, which requires a darkened room to read smears. Conventional FM relies on expensive mercury vapour lamps that have a limited life span, generate large amounts of heat, and are a safety hazard if broken.

https://stoptb.org/wg/gli/assets/documents/TBLabDiagnosisSputum%20Microscopy_Handbook.pdf Accessed: September 1, 2022

AFB smear versus TB NAAT

	AFB Smear +	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)

Improvements of staining

Researchers at the Hartford Hospital in Hartford, Connecticut, compared the rhodamine-auramine staining performed at room temperature versus 37°C and found that the yield of positive smears and the number of bacilli per slides increased for *M. tuberculosis* and for *M. avium* complex when 782 clinical specimens stained at 37°C were compared with the conventional method at room temperature.

McCarter. J Clin Microbiol 1994 *Detection of acid-fast bacilli in concentrated primary specimen smears stained with rhodamine-auramine at room temperature and at 37 degrees C.*

Currently, at the University of Florida Health in Jacksonville, Florida, the auramine-rhodamine bottle is prewarmed in an ambient air 35°C incubator for 30 minutes before use (McCarter YS, Personal Communication, May 29, 2020).

Improvements of staining

Researchers at Johns Hopkins Medical Institutions in Baltimore, Maryland, compared a rapid auramine O fluorescent stain, which required 6 steps and 2 minutes to complete, with the conventional protocol, which requires 8 steps and 22 minutes to complete. Organisms included in the study were *M. tuberculosis*, *M. avium*, and *M. fortuitum*. The 2-minute method outperformed the standard method. Bacilli seemed to be brighter with the shorter method, whereas background debris fluorescence was markedly reduced. Also, the decrease in background fluorescence made the detection of AFB easier.

Hendry. J Clin Microbiol 2009 *Evaluation of a rapid fluorescent staining method for detection of mycobacteria in clinical specimens.*

Improvements of staining

Laboratory scientists at the Public Health Ontario Laboratory, one of the busiest mycobacteriology laboratories in North America with more than 200 AFB smears read per day, provided another example of how to improve fluorescent staining:

They compared an in-house developed bulk container staining method incorporating an acetone rinse step with the standard auramine O/rhodamine B staining method using individual slide racks. Slides stained with acetone were consistently rated of better quality with less background debris and more intensely fluorescing bacilli. *M. avium* yield increased from 53 AFB smear-positive slides with individual rack staining to 60 of 66 (10.6% increase) with acetone rinse staining. The results for *M. tuberculosis* positive slides were about the same, with 47 and 48 AFB smear-positive slides out of 50, respectively.

This finding is significant given that *M. avium* complex organisms are typically smaller and more prone to masking from background fluorescence than *M. tuberculosis*. This factor is especially true in North America, where many mycobacteriology laboratories experience more culture-positive respiratory samples owing to NTM than *M. tuberculosis*.

May. Diagn Microbiol Infect Dis 2018. *A method for improved fluorescent staining for acid fast smear microscopy by incorporating an acetone rinse step.*

Improvements of staining

It is important to remember that NTM, especially rapidly growing mycobacteria, are less frequently detected when using a fluorescent stain versus Ziehl-Neelsen. The American Society for Microbiology Manual of Clinical Microbiology states: “If the presence of a rapid grower is suspected and the results of acid-fast stains, in particular fluorochrome stains, are negative, it may be worthwhile to stain the smear with carbol fuchsin and to use a weaker decolorizing process.”

Manual of Clinical Microbiology 2019. *Mycobacterium: general characteristics, laboratory detection, and staining procedures.*

"Proof-Of-Concept" Evaluation of an Automated Sputum Smear Microscopy System for Tuberculosis Diagnosis

James J. Lewis[1,2*], Violet N. Chihota[2], Minty van der Meulen[2], P. Bernard Fourie[3], Katherine L. Fielding[1], Alison D. Grant[1], Susan E. Dorman[4], Gavin J. Churchyard[1,2,5]

1 London School of Hygiene and Tropical Medicine, London, United Kingdom, **2** Aurum Institute, Johannesburg, South Africa, **3** University of Pretoria, Pretoria, South Africa, **4** Johns Hopkins University, Baltimore, Maryland, United States of America, **5** School of Public Health, University of Witwatersrand, Johannesburg, South Africa

Abstract

Background: "TBDx" is an innovative smear microscopy system that automatically loads slides onto a microscope, focuses and digitally captures images and then classifies smears as positive or negative using computerised algorithms.

PLoS One. 2012; 7(11): e50173.
Published online 2012 Nov 29.

Deep learning / artificial intelligence

- **Deep Learning for Chest Radiograph Diagnosis in the Emergency Department. Hwang. (South Korea) Radiology. 2019:**

A deep learning algorithm used with emergency department chest radiographs showed diagnostic performance for identifying clinically relevant abnormalities and helped improve the sensitivity of radiology residents' evaluation.

- **Automated Interpretation of Blood Culture Gram Stains by Use of a Deep Convolutional Neural Network. Smith KP, Kang AD, Kirby JE. J Clin Microbiol. 2018:**

Microscopic interpretation of stained smears is one of the most operator-dependent and time-intensive activities in the clinical microbiology laboratory. Here, we investigated application of an automated image acquisition and convolutional neural network (CNN)-based approach for automated Gram stain classification.

***Taken together**, our data support a proof of concept for a fully automated classification methodology for blood-culture Gram stains. Importantly, the algorithm was highly adept at identifying image crops with organisms and could be used to present prescreened, classified crops to technologists to accelerate smear review.*

Slide reading and artificial intelligence

Xiong and colleagues built a convolutional neural network (CNN) model specifically to recognize *M. tuberculosis* complex organisms in formalin-fixed paraffin-embedded tissue blocks. The training set contained 45 samples (30 positive cases and 15 negative cases according to 2 pathologists' readings). Upon training the neural network model, 201 samples (108 positive cases and 93 negative cases) were collected as a test set to challenge the model. The artificial intelligence-assisted detection method achieved 97.9% sensitivity and 83.7% specificity.

Xiong. J Thorac Dis 2018. *Automatic detection of mycobacterium tuberculosis using artificial intelligence*

Machine learning

Horvath. Tuberculosis (Edinburgh) 2020. Machine-assisted interpretation of auramine stains substantially increases through-put and sensitivity of microscopic tuberculosis diagnosis.

The authors validated a scanning and analysis system that combines fully-automated microscopy with deep-learning based image analysis. After automated scanning, the system summarizes diagnosis-relevant image information and presents it to the microbiologist in order to assist diagnosis. They tested the benefit of the automated scanning and analysis system using 531 slides from routine workflow, of which 56 were from culture positive specimen. Assistance by the scanning and analysis system allowed for a higher sensitivity (40/56 positive slides detected) than manual microscopy (34/56 positive slides detected), while greatly reducing manual slide-analysis time from a recommended 5-15 min to around 10 s per slide on average.

AFB smear & infection prevention

Seventy-five percent of all people who are acid-fast bacillus (AFB) sputum smear positive will remain so for at least 2 weeks, with the majority remaining positive for 4 to 6 weeks. Therefore, while it is realized that ***it is generally not practical or necessary to keep all patients hospitalized until 3 consecutive sputum smear are negative***, other considerations must be evaluated.

The risk of transmission from a person with TB on appropriate therapy showing clinical improvement (reduction of cough, fever, and AFB on smear; and improvement in chest X-ray) is substantially reduced after 2 weeks on therapy.

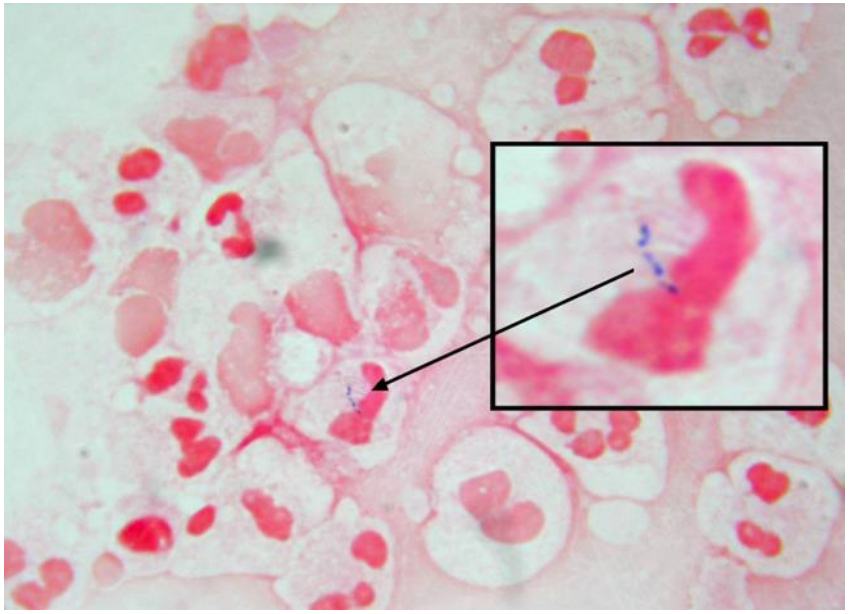
https://www.sfcdcp.org/tb-control/tuberculosis-information-for-medical-providers/hospital-discharge-of-tuberculosis-patients-and-suspects/ Accessed September 1, 2022

Gram-labile rods

Anecdotal - more than 25 years ago

Sputum sent for bacteriologic examination

Gram stain: Leucocytes, Gram-positive and Gram-negative rods, Gram-positive cocci, and a few Gram-labile rods



Gram-labile rods

Anecdotal - more than 25 years ago

Sputum sent for bacteriologic examination
Gram stain: Leucocytes, Gram-positive and Gram-negative rods, Gram-positive cocci and a few Gram-labile rods

→ **Agar plates – next morning and Day 2**
Normal flora
However, no colonies which could be the Gram-labile rods

Med Tech had a suspicion and did a Ziehl-Neelsen stain:
AFB positive

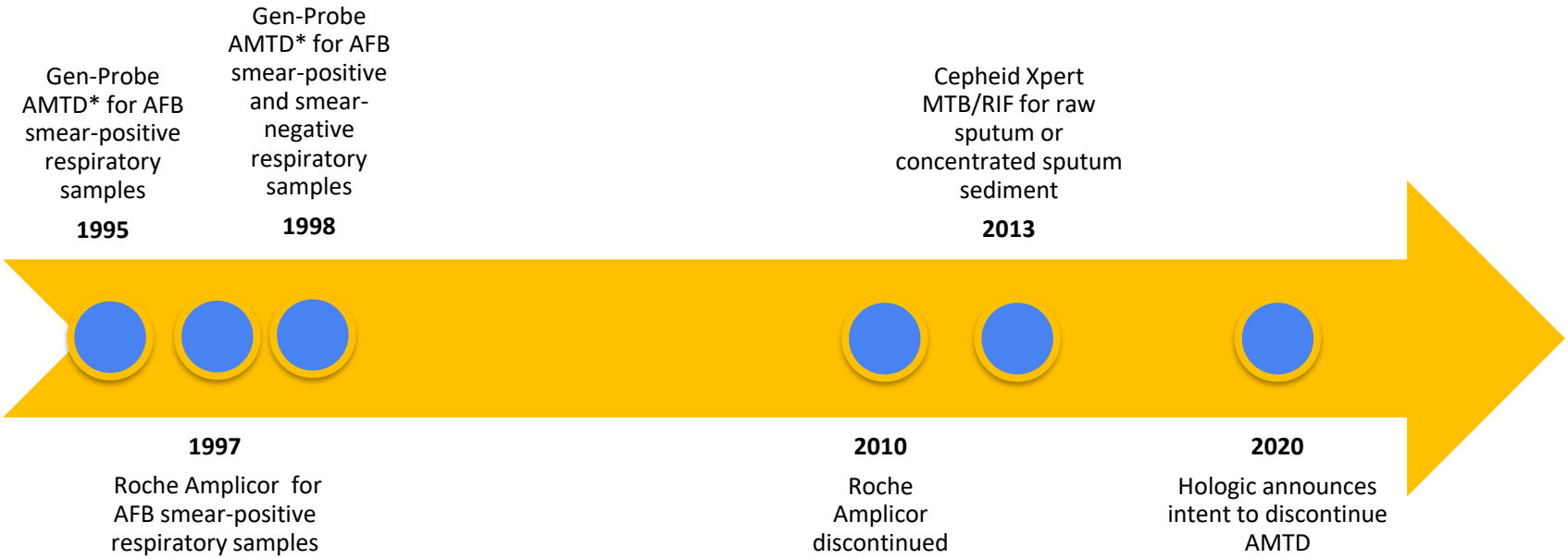
Who has a similar story?

A new pulmonary TB patient was diagnosed!

Topics

- State of the TB epidemic
- Quantum leaps in mycobacteriology during last 50 years
- AFB stain - still relevant?
- **TB NAAT – still a frontier?**
- Systems
- Closing remarks

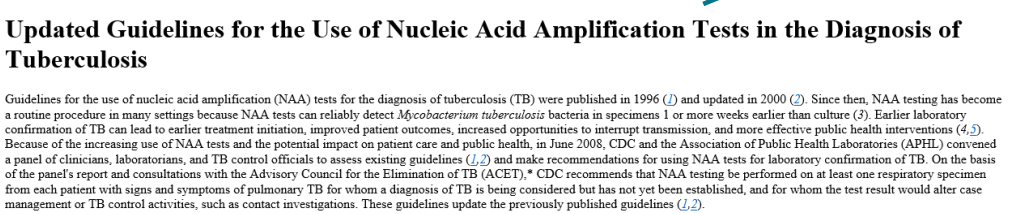
History of commercially-available NAA tests for MTB in United States



*Gen-Probe now Hologic

Nucleic Acid Amplification (NAA) Testing

- Critical diagnostic tool for rapid detection of *M. tuberculosis*
- CDC updated NAA test guidelines in 2009



Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

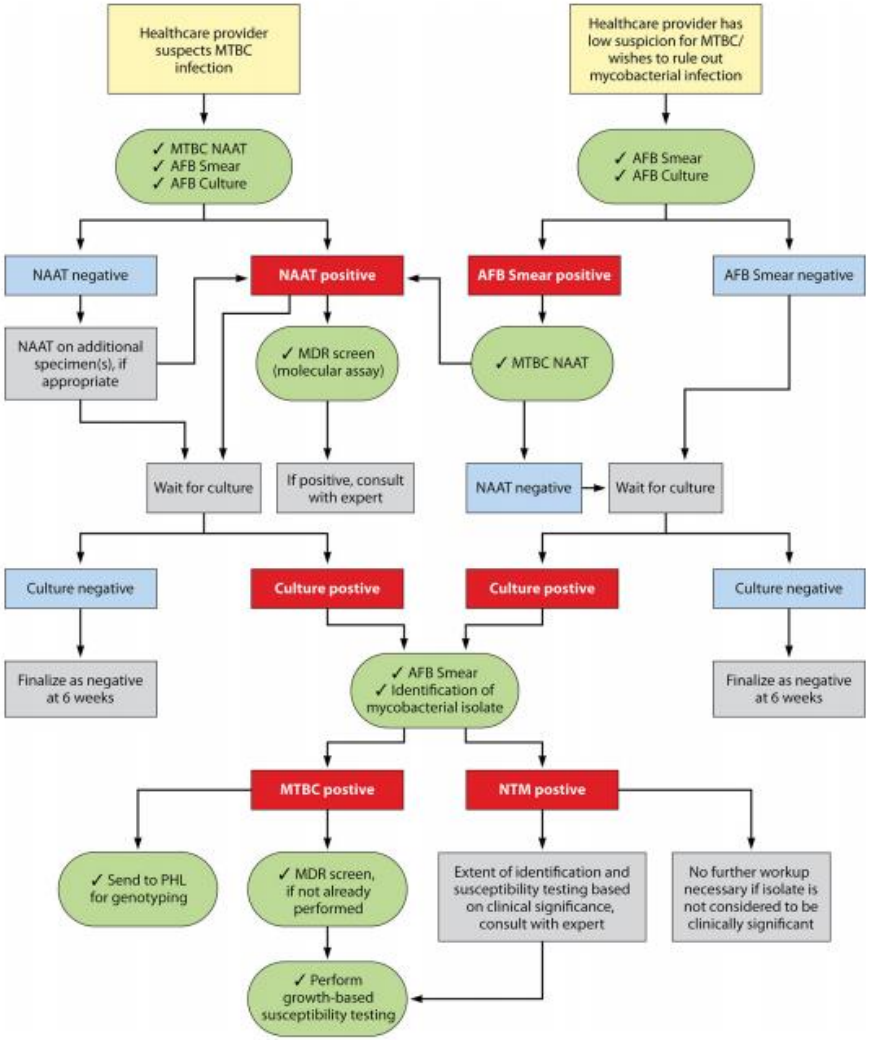
Guidelines for the use of nucleic acid amplification (NAA) tests for the diagnosis of tuberculosis (TB) were published in 1996 (*1*) and updated in 2000 (*2*). Since then, NAA testing has become a routine procedure in many settings because NAA tests can reliably detect *Mycobacterium tuberculosis* bacteria in specimens 1 or more weeks earlier than culture (*3*). Earlier laboratory confirmation of TB can lead to earlier treatment initiation, improved patient outcomes, increased opportunities to interrupt transmission, and more effective public health interventions (*4,5*). Because of the increasing use of NAA tests and the potential impact on patient care and public health, in June 2008, CDC and the Association of Public Health Laboratories (APHL) convened a panel of clinicians, laboratorians, and TB control officials to assess existing guidelines (*1,2*) and make recommendations for using NAA tests for laboratory confirmation of TB. On the basis of the panel's report and consultations with the Advisory Council for the Elimination of TB (ACET),* CDC recommends that NAA testing 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, such as contact investigations. These guidelines update the previously published guidelines (*1,2*).

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.

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm

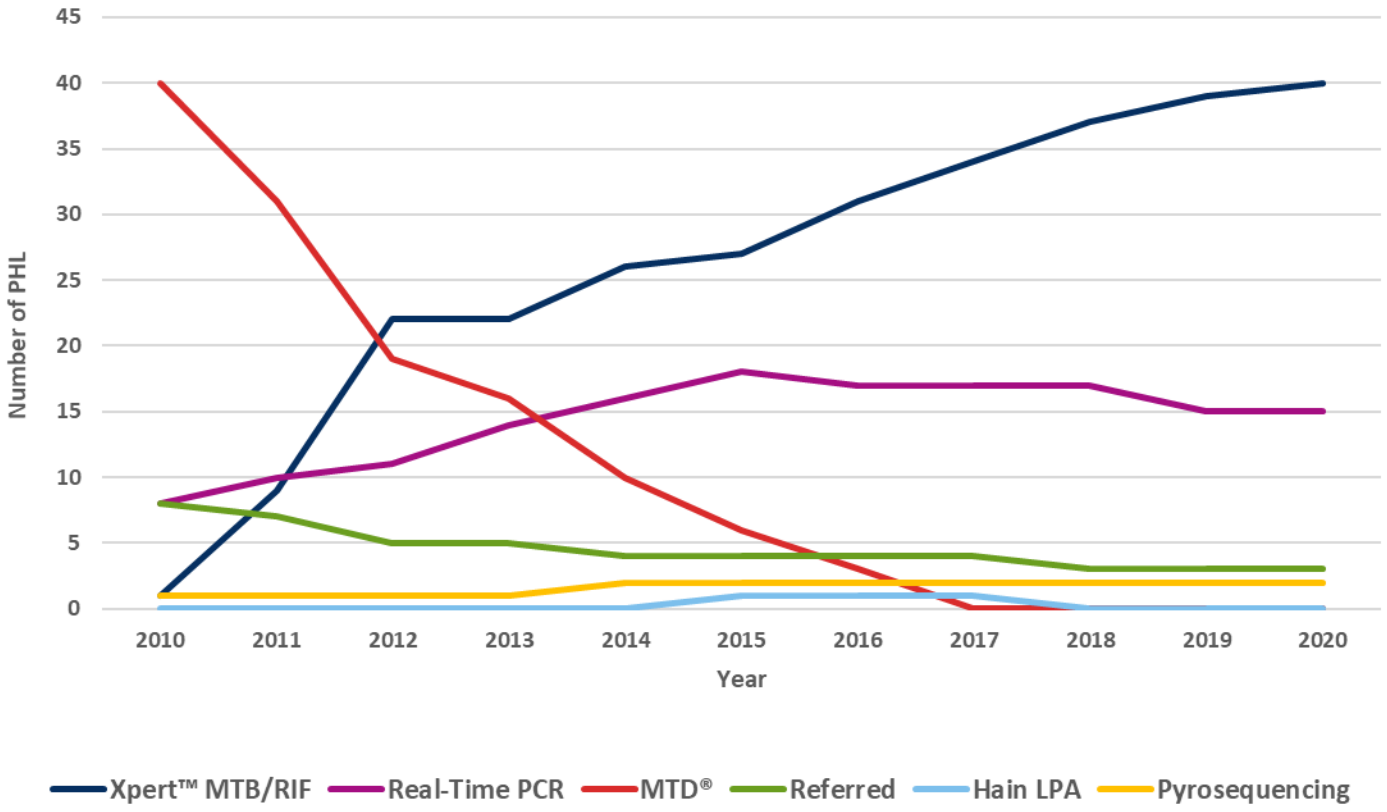
Proposed ideal algorithm for Mycobacteriology Testing

- Algorithm proposed in Clinical Microbiology Reviews (2018)



https://cmr.asm.org/content/cmr/31/2/e00038-17.full.pdf

Changes in NAA test methods among PHL, 2010–2020



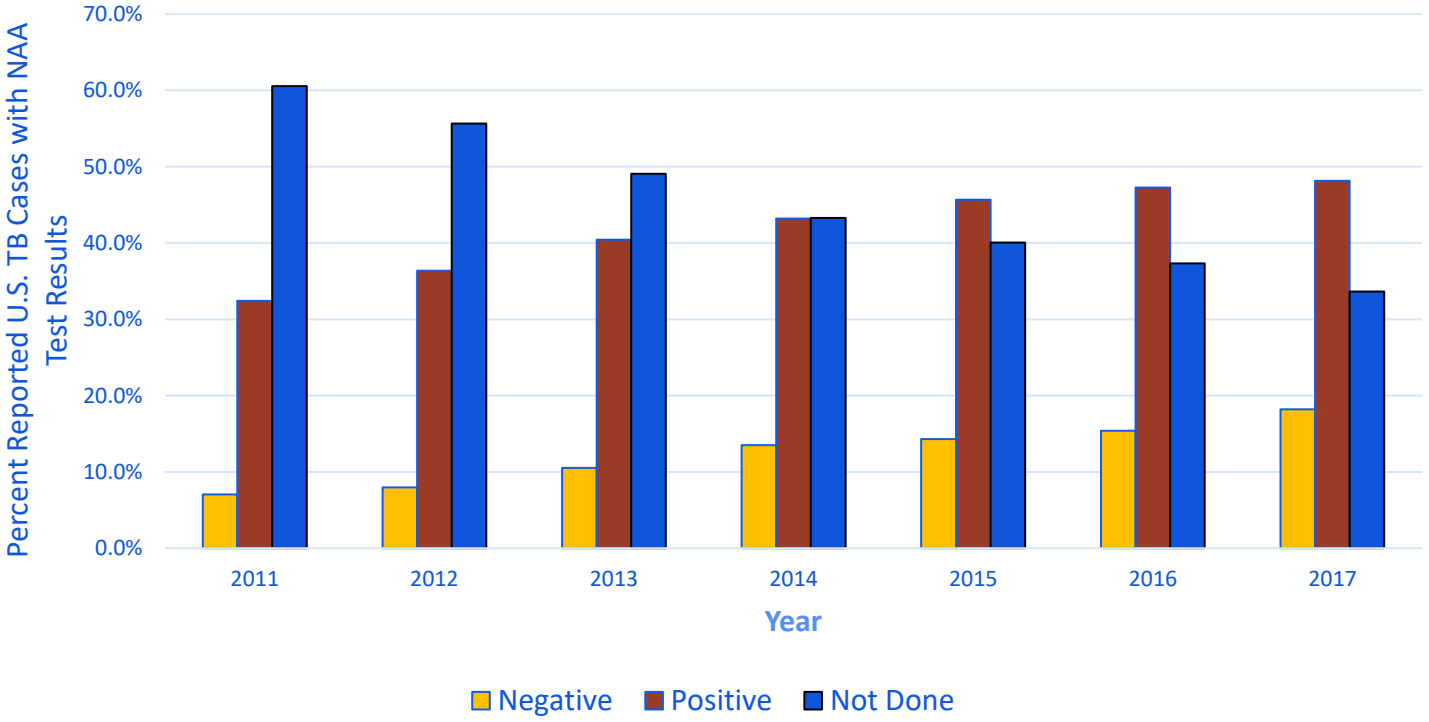
Frequency of NAA tests performed among TB cases 2011–2017

NAA Test Result	Frequency	Percent
Positive	27, 912	41.6
Negative	8, 215	12.2
Indeterminate	135	0.2
Not done/UNK	30, 820	45.9
Total	**67, 082**	**100**

- Of those patients with a reported NAA test result, 74.9% had sputum tested
- Among patients with other specimen types tested, 58.3% had lymph node, bronchial fluid, and lung tissue examined

Use of NAA tests 2011–2017

- **Use should be standard of care for those presumed to have TB (CDC guidelines) but continued progress needed**



What factors were associated with having NAA test performed?

- Positive AFB smear [OR 4.0 (95% CI: 3.87–4.13)]
- Positive culture [OR 2.39 (95% CI: 2.30–2.49)]

AFB Smear Result	NAA Test Performed	NAA Test Not Performed
Positive	70.9% (24,093/ 33,937)	29.0% (9,844/ 33,937)
Negative	38.0% (11,490/ 30,244)	62.0% (18,754/ 30,244)

Culture Result	NAA Test Performed	NAA Test Not Performed
Positive	59.8% (31,028/ 51,909)	40.2% (20,881/ 51,909)
Negative	38.3% (4,851/ 12,657)	61.7% (7,806/ 12,657)

What factors were associated with having a positive NAA test result?

- Positive AFB smear [OR 13.17 (95% CI: 12.41–13.98)]
- Positive culture [OR 27.75 (95% CI: 25.65–30.03)]

AFB Smear Result	NAA Test Positive	NAA Test Negative
Positive	92.1% (22,109/ 24,005)	7.9% (1,896/ 24,005)
Negative	47.0% (5,358/ 11,409)	53.0% (6,051/ 11,409)

Culture Result	NAA Test Positive	NAA Test Negative
Positive	84.5% (26,775/ 31,679)	15.5% (4,904/ 31,679)
Negative	19.1% (920/ 4,824)	80.9% (3,904/ 4,824)

Open Forum Infectious Diseases 2021

Open Forum Infectious Diseases

MAJOR ARTICLE

Use of Nucleic Acid Amplification Testing for Rapid Detection of *Mycobacterium tuberculosis* Complex Among US Tuberculosis Patients, 2011–2017

Varsha Kumar,[1,a] Tracy L. Dalton,[1] Lori R. Armstrong,[1,b] Amy Whitesell,[1,c] Rongxia Li,[1] and Angela M. Starks[1]

[1]Division of Tuberculosis Elimination, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Background. Nucleic acid amplification (NAA) tests rapidly detect *Mycobacterium tuberculosis* complex directly from clinical specimens, providing valuable results for those evaluated for tuberculosis.

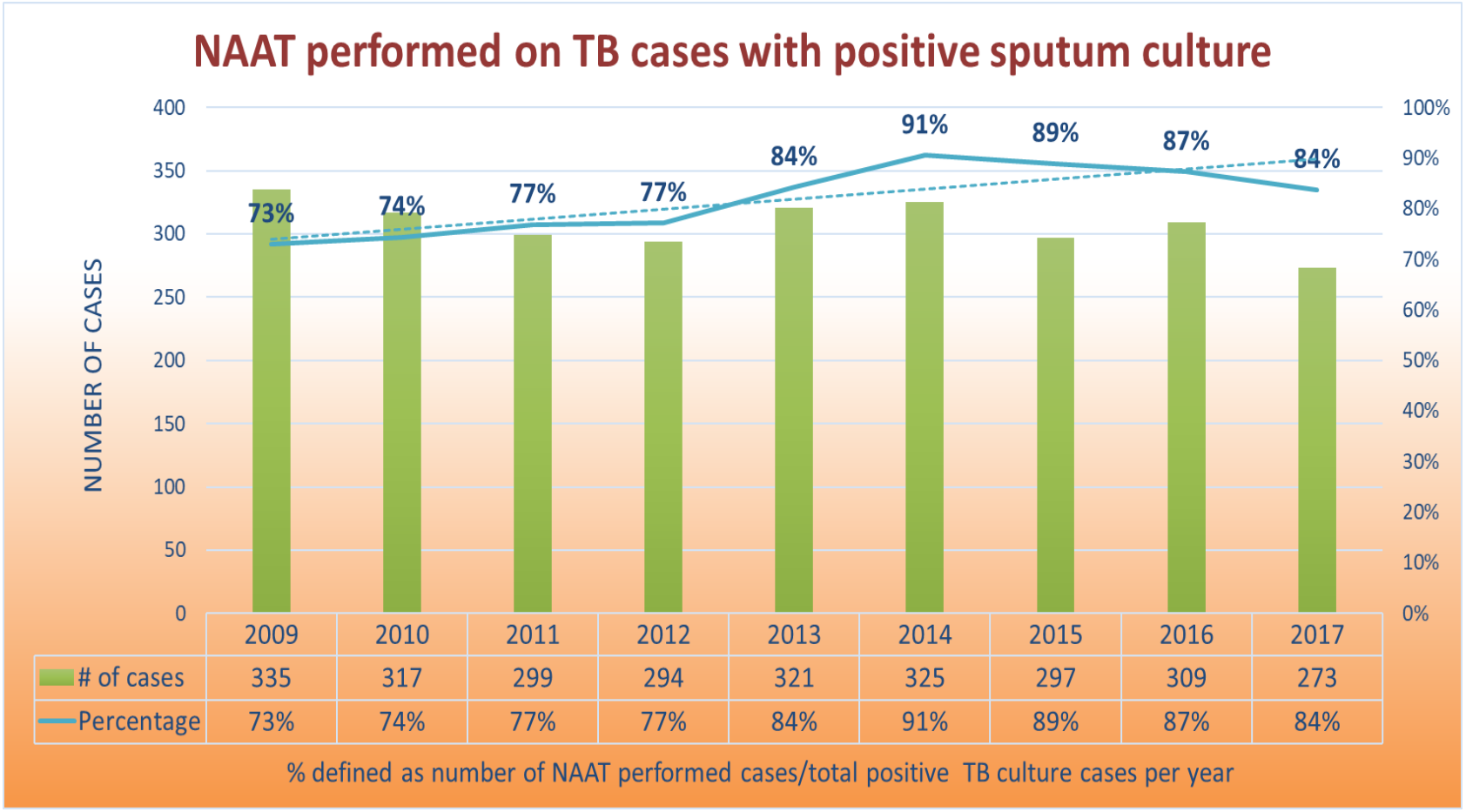
Methods. We analyzed characteristics of cases with NAA testing performed, compared cases with positive and negative NAA test results, and calculated turnaround time and time to treatment for all verified cases reported to the National Tuberculosis Surveillance System in the United States during 2011–2017.

Results. Among 67 082 verified tuberculosis cases with NAA testing information, 30 820 (45.9%) were reported as not having an NAA test performed; the proportion without NAA testing declined annually, from 60.5% in 2011 to 33.6% in 2017. Of 67 082 verified cases, 27 912 (41.6%) had positive, 8215 (12.2%) had negative, and 135 (0.2%) had indeterminate NAA test results. Among the 33 937 cases with an acid-fast bacilli (AFB) smear-positive result, 24 093 (70.9%) had an NAA test performed; 11 490 of the 30 244 (38.0%) with an AFB smear-negative result had an NAA test performed. Although sputum was the most common specimen type tested, 79.8% (7023/8804) of nonsputum specimen types had a positive NAA test result. Overall, 63.7% of cases with laboratory testing had NAA test results reported <6 days following specimen collection; for 13 891 cases not yet on treatment, median time to treatment after the laboratory report date was 2 days.

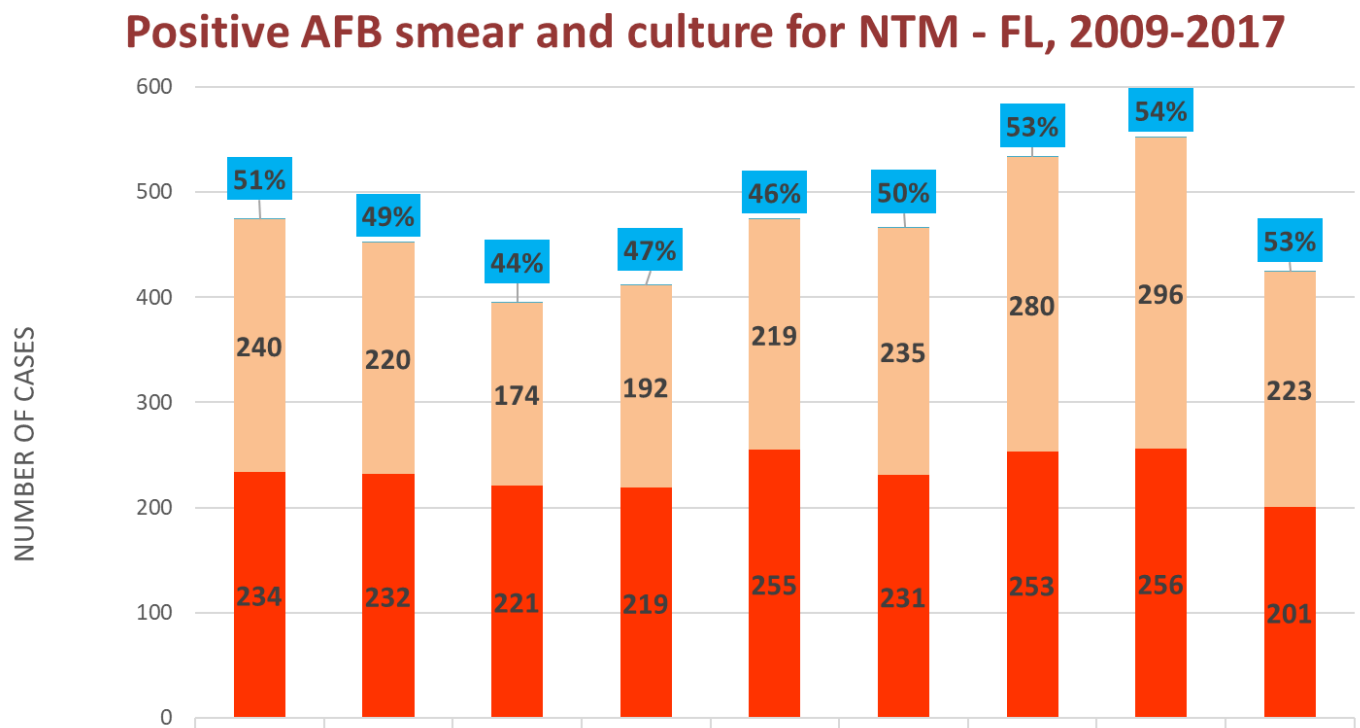
Conclusions. Our analyses demonstrate increased NAA test utilization between 2011 and 2017. However, a large proportion of cases did not have an NAA test performed, reflecting challenges in broader uptake, suggesting an opportunity to expand use of this diagnostic methodology.

Keywords. nucleic acid amplification testing; NAA; tuberculosis.

Florida data



Florida data



	2009	2010	2011	2012	2013	2014	2015	2016	2017
% AFB positive NTM	51%	49%	44%	47%	46%	50%	53%	54%	53%
Positive NTM culture	240	220	174	192	219	235	280	296	223
Positive TB culture	234	232	221	219	255	231	253	256	201

% defined as number of AFB positive NTM cases/total AFB smear cases (NTM and TB) per year

California – TB verification criteria

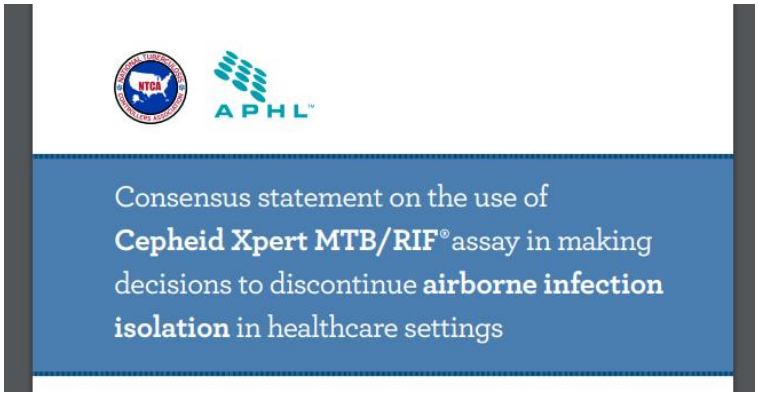
	2016 (No.)	2016 (%)	2017 (No.)	2017 (%)	2018 (No.)	2018 (%)	2019 (No.)	2019 (%)	2020 (No.)	2020 (%)
All Cases	2059	100.0	2057	100.0	2097	100.0	2112	100.0	1705	100.0
Verification Criteria										
Positive Culture	1693	82.2	1713	83.3	1722	82.1	1767	83.7	1377	80.8
Positive Nucleic Acid Amplificaton	44	2.1	42	2.0	65	3.1	52	2.5	66	3.9
Positive Smear/Tissue	7	0.3	7	0.3	9	0.4	5	0.2	7	0.4
Clinical Case Definition	239	11.6	218	10.6	237	11.3	207	9.8	185	10.9
Verified by Provider Diagnosis	76	3.7	77	3.7	64	3.1	81	3.8	70	4.1

California – NAAT
2016 - 2020

Nucleic Acid Amplification Test										
Positive	1015	49.3	982	47.7	1075	51.3	1083	51.3	934	54.8
Negative	302	14.7	397	19.3	463	22.1	488	23.1	338	19.8
Not Done	737	35.8	672	32.7	552	26.3	536	25.4	432	25.3
Indeterminate	2	0.1	2	0.1	2	0.1	1	0.0	.	.
Unknown	3	0.1	4	0.2	5	0.2	4	0.2	1	0.1

Use of NAA testing results to guide decision making in use of airborne infection isolation (A.I.I.)

- **February 2015, U.S. FDA approved expanded claims for Xpert MTB/RIF related to A.I.I.**
- **National TB Controllers Association and Association of Public Health Laboratories issued guidance in 2016**
- **Based on negative results from 1 or 2 sputum specimens predictive of results of 2 or 3 AFB smears being negative**
  - Sputum test results alone should NOT be only criteria for decision making



http://www.tbcontrollers.org/docs/resources/NTCA_APHL_GeneXpert_Consensus_Statement_Final.pdf

Decision to discontinue airborne infection isolation in healthcare settings

Interpretation of an Xpert result must be made in the context of the clinical and radiographic presentation and the clinician's suspicion for infectious TB. **A decision to remove a patient with a negative Xpert result from All must consider the clinical presentation and the risk of possible transmission of TB from an infectious patient to others. Such a decision should not be based on sputum test results alone.** The sensitivity of sputum testing for TB is subject to variability from a variety of factors, including sampling (e.g., poor specimen quality), inappropriate transport and processing of the specimen, errors in performance of the assay itself, and errors in labelling or reporting.

NTCA/APHL GeneXpert Consensus Statement – April 2016

San Francisco study

- In a prospective cohort study with a pragmatic, before-and-after implementation design, the authors analyzed 621 consecutive hospitalized patients undergoing sputum examination for evaluation of active pulmonary TB from January 2014 to January 2016 at the **Zuckerberg San Francisco General Hospital and Trauma Center**.

JAMA Intern Med. 2018; 178(10):1380-1388

San Francisco study

- The mean hospital costs per molecular TB test-negative patient decreased from $46,921 to $33,574 after implementation of the algorithm, providing an **average savings of $13,347 per patient.**
- The authors estimated utilization and costs for approximately 250 patients completing TB evaluation each year and projected a total **annual savings to the hospital of $3.3 million**.

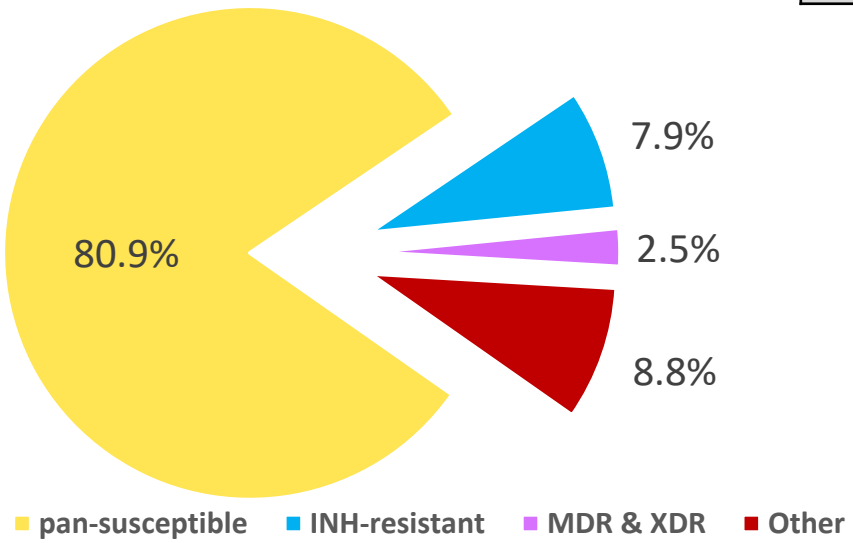
Don’t wait for the magic bullet

Improving the current system:

- Improve the use of NAA test
- Universal MDR-TB screening when NAA test positive
- Take advantage of electronic ordering capabilities and implement TB related algorithm

6+ years of clinical TB WGS testing

Resistance in TB, NYS 2016-2020



WGS Performance Characteristics with Culture-based AST as the Gold Standard

	EMB	FLQ	INH	PZA	RIF	SM	KAN	ETH	ALL
Susceptibility-PV	0.99	0.99	0.99	0.98	1.00	0.95	1.00	0.94	0.98
n	1516	343	1522	1518	1516	1178	326	334	8253

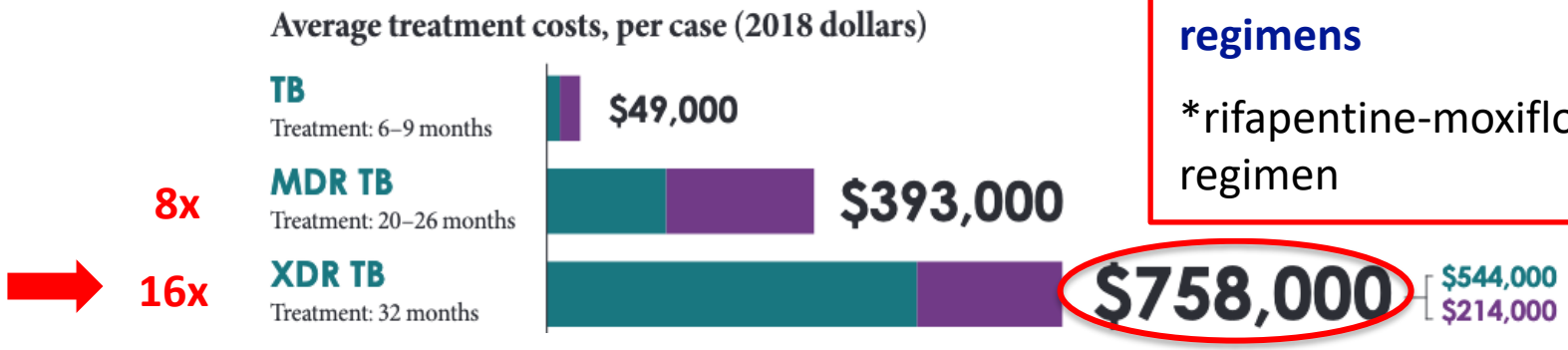
First-line RIPE in blue

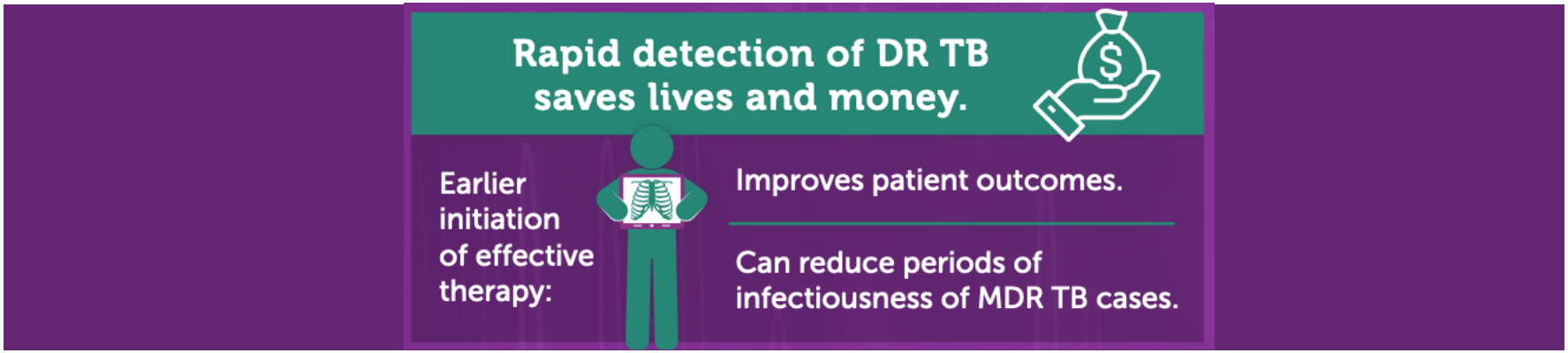
NEW YORK STATE Department of Health | Wadsworth Center

Drug Resistant Tuberculosis

New 4- and 6- month TX regimens

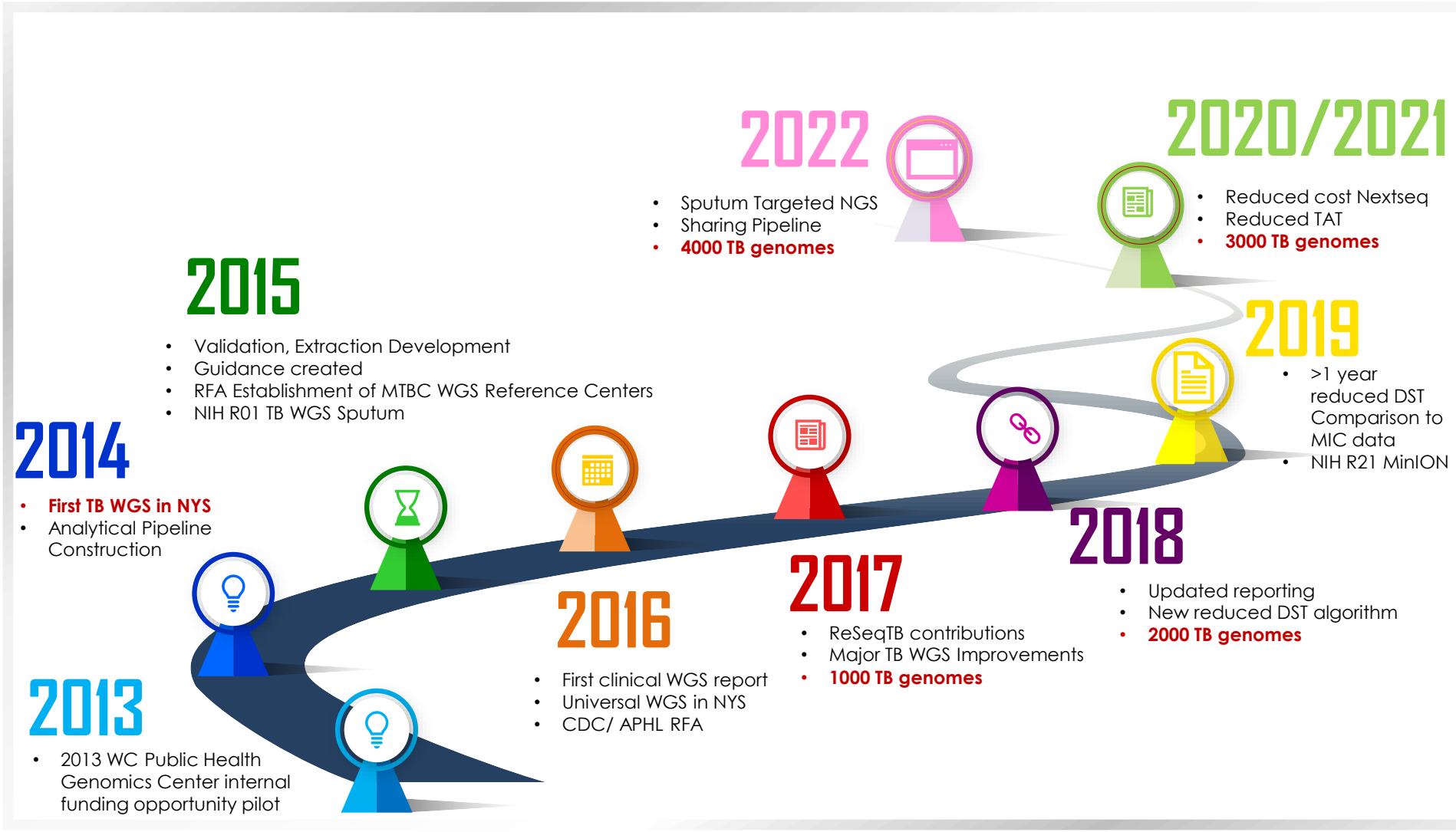
*rifapentine-moxifloxacin regimen





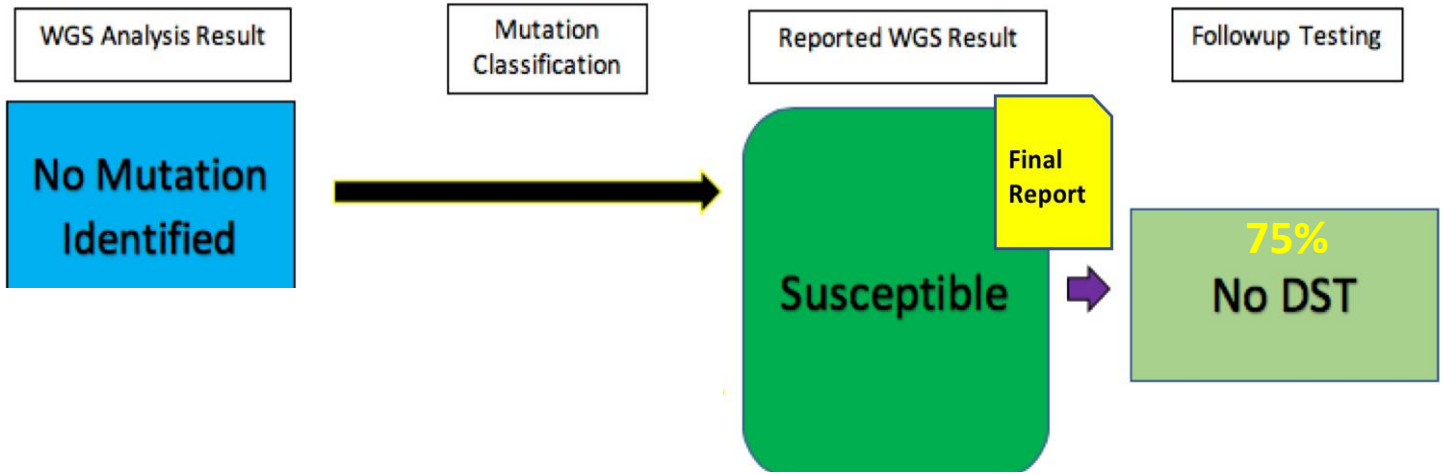
https://www.cdc.gov/nchhstp/newsroom/docs/factsheets/costly-burden-dr-tb-508.pdf;
https://www.cdc.gov/tb/publications/infographic/pdf/tbMDDR508.pdf

TB WGS Timeline



NEW YORK STATE | Department of Health | Wadsworth Center

2 ½ years later - New algorithm for phenotypic AST



10 states have been funded to do clinical WGS in the newest ELC grant update: California, Georgia, Iowa, Michigan, New York, Ohio, Texas, Virginia, Washington, Wisconsin

Targeted next-generation sequencing: a Swiss army knife for mycobacterial diagnostics? – ERJ 2021

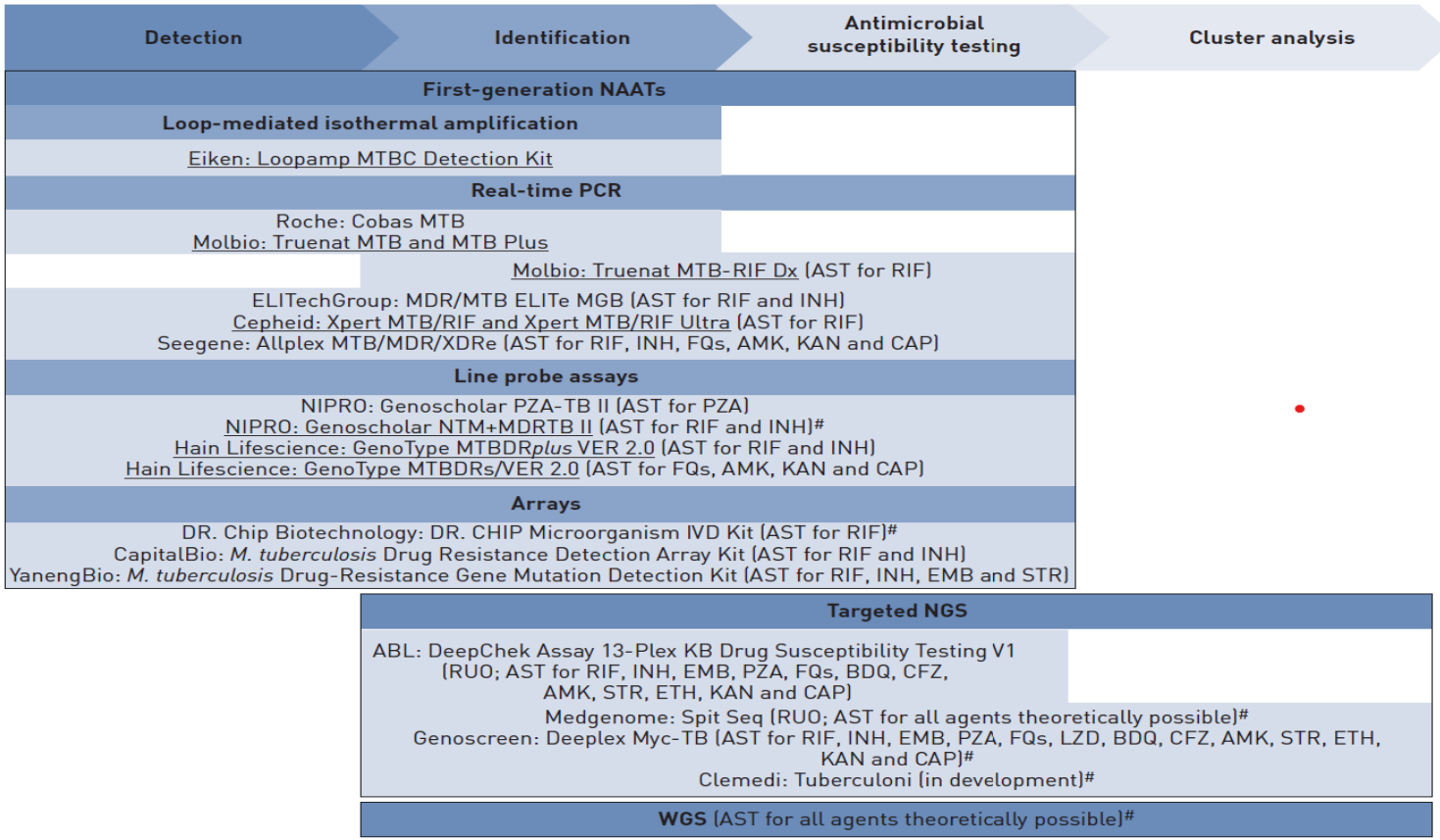


FIGURE 1 Functionality of selected first-generation nucleic acid amplification tests (NAATs) for *Mycobacterium tuberculosis* complex (MTBC) diagnostics compared with targeted next-generation sequencing (NGS) and whole genome sequencing (WGS). All currently World Health Organization-endorsed NAATs are listed and underlined [10]. Additional assays are included but many more exist. Unless otherwise marked (#), the assays are only capable of identifying MTBC rather than differentiating nontuberculous mycobacteria. Cluster analysis is not currently a feature of Spit Seq but could be integrated and the resolution of Deeplex is considerably lower than WGS in this context. AMK: amikacin; AST: antimicrobial susceptibility testing; BDQ: bedaquiline; CAP: capreomycin; CFZ: clofazimine; EMB: ethambutol; ETH: ethionamide; FQs: fluoroquinolones; INH: isoniazid; KAN: kanamycin; LZD: linezolid; PZA: pyrazinamide; RIF: rifampicin; RUO: research use only; STR: streptomycin.

Topics

- State of the TB epidemic
- Quantum leaps in mycobacteriology during last 50 years
- AFB stain - still relevant?
- TB NAAT – still a frontier?
- **Systems**

Aspen grove – a system!

Faster turnaround times

In 1992, survey among all NYS permitted TB laboratories (>150):

- BACTEC-AccuProbe-BACTEC combo applied in <20% of all TB samples tested in NYS
- TB/AST was only performed when there was a suspicion for MDR-TB
- Many TB laboratories worked only Monday through Friday

The model NYS Fast Track program for TB testing was born

NYS Fast Track

- **State-of-the-art** TB laboratory procedures for highly infectious AFB smear positive patients
- **Rapid specimen transport** by overnight courier to a central laboratory
- For **NTM patients**, period of exposure to potentially toxic and unnecessary anti-TB drugs dramatically shortened; these patients can also be released from AII earlier
- Results are **transmitted via fax and mail** to the sender and reported immediately to TB Control
- Mail report is accompanied by **current information on case management**
- **Implementation of new procedures** is usually time consuming and costly. Upon availability, new technologies are quickly incorporated into the Fast Track program, thus providing immediate benefits to all enrolled hospitals, clinics and medical centers.

NYS Fast Track program updates

1996 - April: Nucleic acid amplification test implemented (MTD)

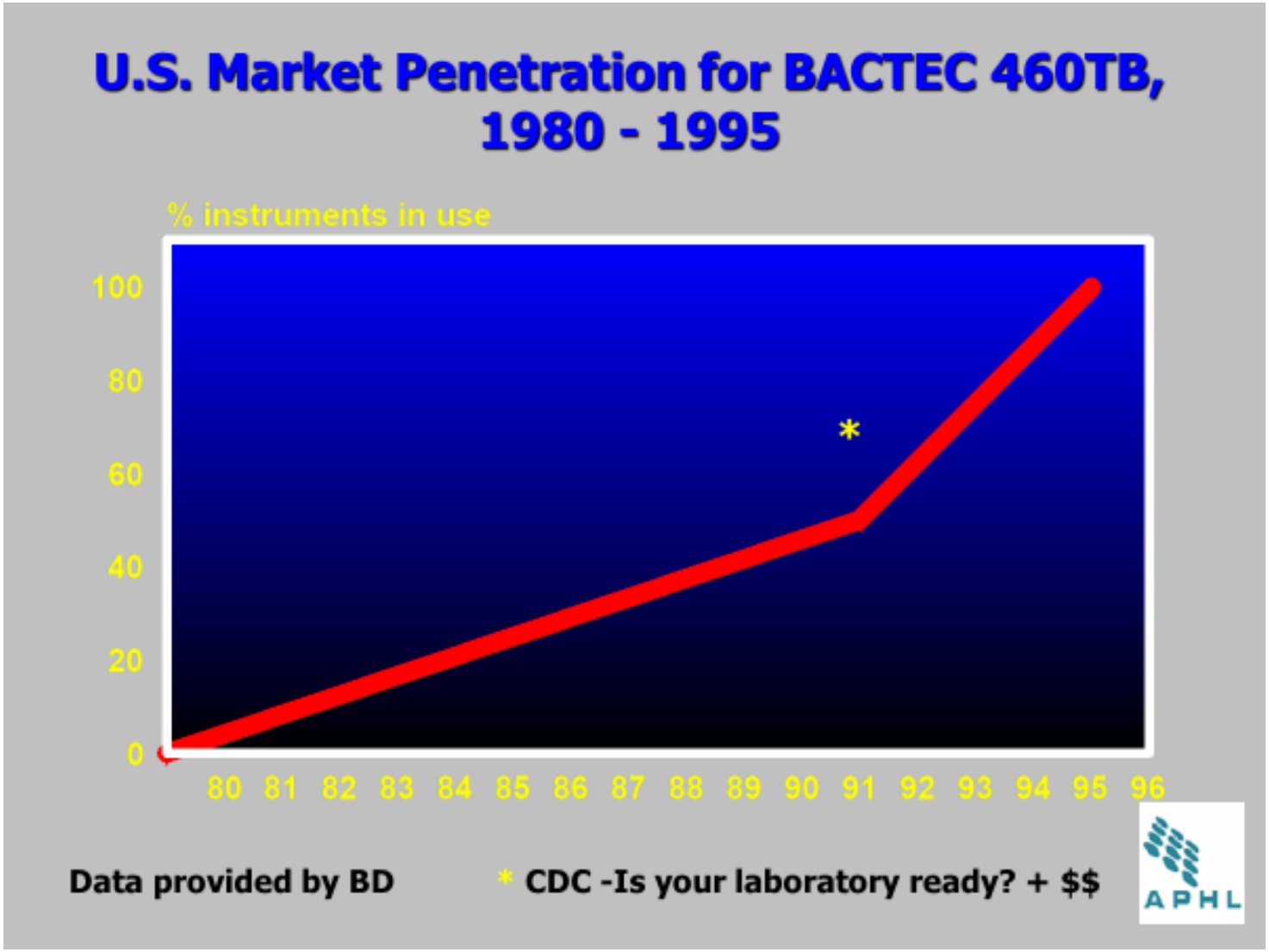
1999 - *rpoB* gene sequencing for rifampin resistance (Sanger)

2002 - Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis.

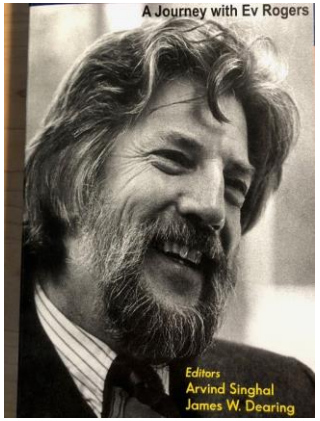
2010 - Combined real-time PCR and *rpoB* gene pyrosequencing for rapid identification of *Mycobacterium tuberculosis* and determination of rifampin resistance directly in clinical specimens.

2016- Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles on Clinical Cases of *Mycobacterium tuberculosis* in New York State.

Market penetration



Everett Rogers



March 6, 1931 – October 21, 2004

An eminent American communication theorist and sociologist, who originated the diffusion of innovations theory and introduced the term *early adopter*.

Rogers' Bio cont'd

- Rogers was born on his family's Pinehurst Farm in Carroll, Iowa, in 1931. He was a self-described "son of the soil" - milking cows, raising pigs and chickens, picking corn, and driving a tractor.
- Attended a one-room schoolhouse 1936-1944.
- Carroll High School 1944-1948

  Member of Future Farmers of America

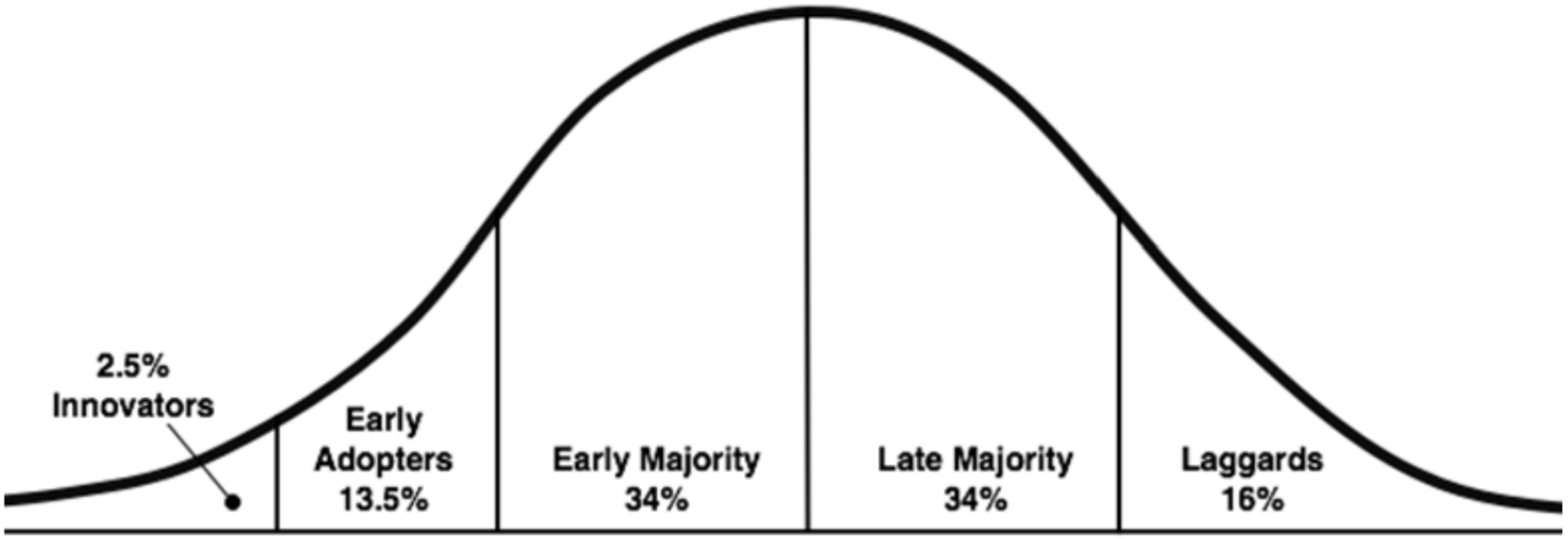
Rogers' Bio cont'd

- Rogers had no plans to attend university (he **planned to be a farmer**) until a schoolteacher drove him and some classmates to **Ames to visit Iowa State University**. Rogers decided to pursue a degree there.
- He received a B.S. in Agriculture in 1952.
- He then served in the Korean War for two years (1952-1954) - entered as a second lieutenant on graduation from college.

Rogers' Bio cont'd

- He returned to **Iowa State University** to earn a M.S. in 1955 and a Ph.D. in 1957, both in Rural Sociology.
- Rogers held faculty positions at **Ohio State University** (1957–63) in the Department of Rural Sociology, studied diffusion of agricultural innovations-he started the position the day after he had his doctoral defense.
- 1962-The first edition of the *Diffusion of Innovations* – **at the age of 31 years**

Adapter categories

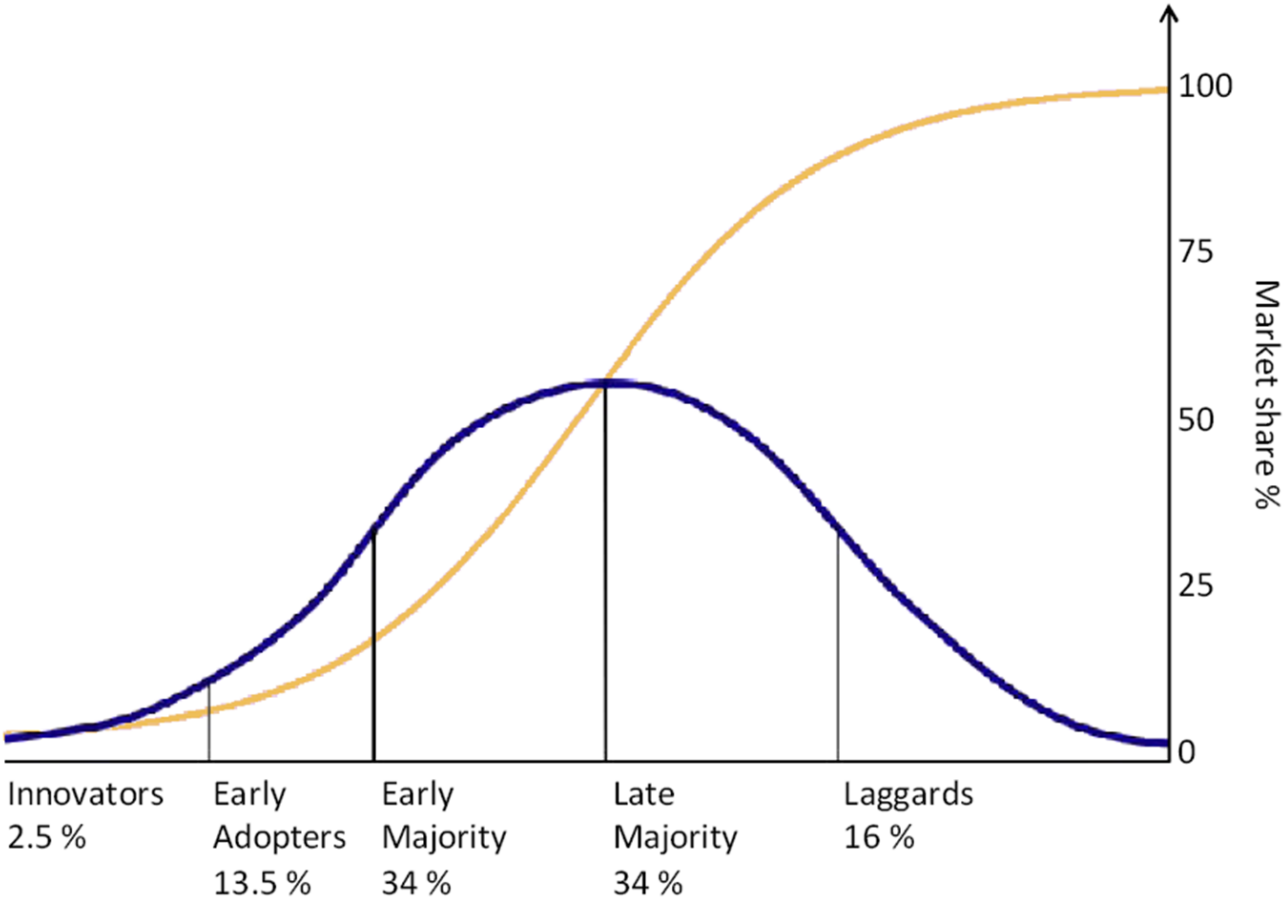


Adopter categories

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

Everett M. Rogers, 1931-2004

Diffusion of innovations



Change is not easy…

- Said is not heard,
- Heard is not understood,
- Understood is not implemented,
- Implemented is not retained.

Topics

- State of the TB epidemic
- Quantum leaps in mycobacteriology during last 50 years
- AFB stain - still relevant?
- TB NAAT – still a frontier?
- Systems
- **Closing remarks**

Summary

- AFB smear is not going away – it is worth improving it
- TB NAAT are underutilized – help in promoting them
- Systems thinking beyond the laboratory is important – tear down the walls…
- Change is not easy – but doable

1992 & 2021 TB cases & incidence

	1992		2021		
CT	156	4.8	54	1.5	3
ME	24	1.9	14	1.0	1
MA	428	7.1	151	2.2	6
NH	18	1.6	12	0.9	1
NY	4,574	25.2	681	3.4	19
RI	54	5.4	17	1.6	1
VT	7	1.2	2	0.3	1
NACMID	5,261		931		5.7
USA	26,673	10.4	7,860	2.4	3.4

TB Elimination 330 cases nationwide [1/million]

Change…

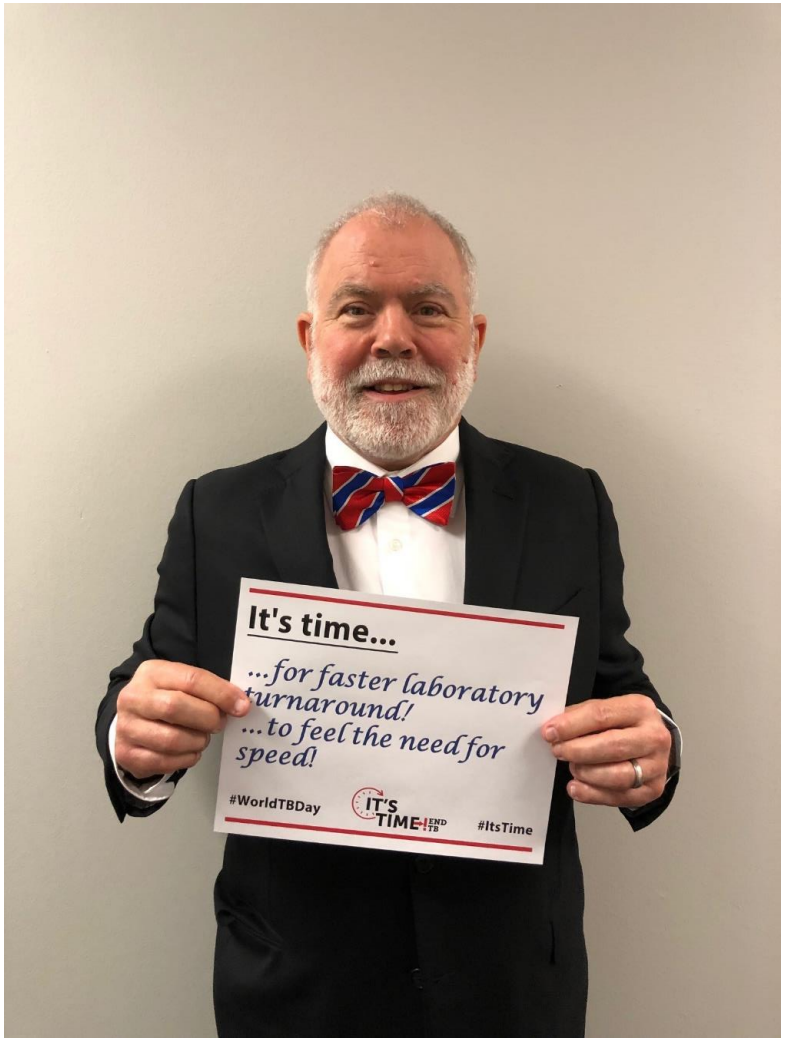
Said is not heard

Heard is not understood

Understood is not implemented

Implemented is not retained

March 24 – World TB Day



A world free of TB

We all aspire to a world free of TB, and health in general for all.

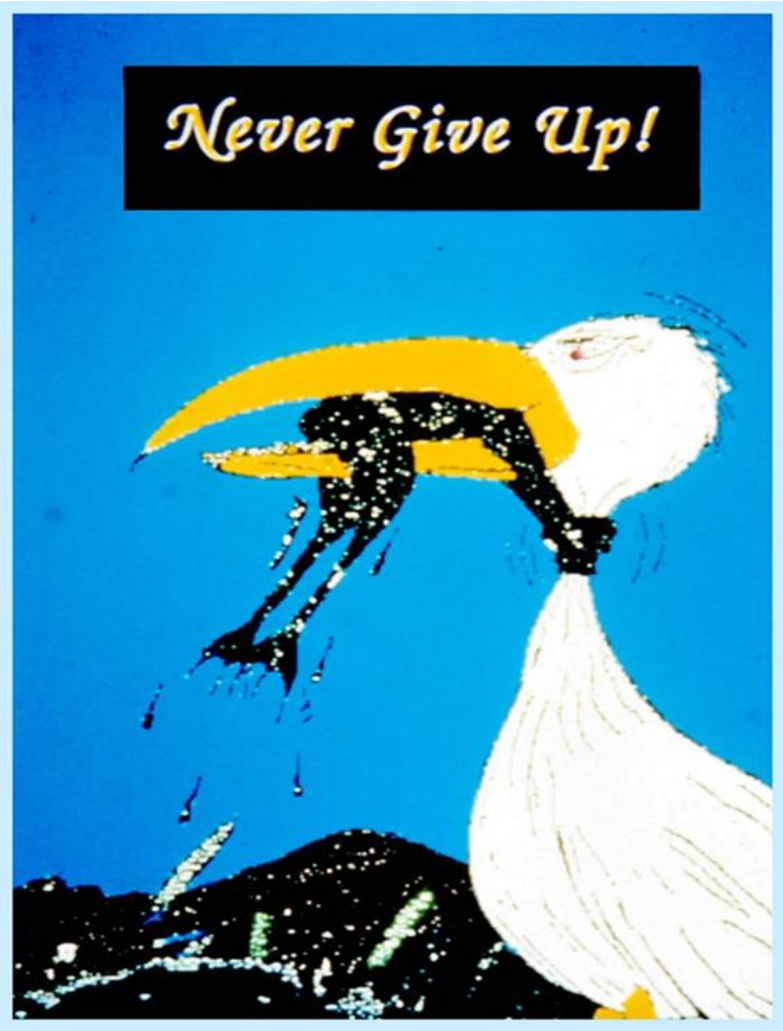
It was **Mahatma Gandhi** who poignantly stated, ***'It is health that is real wealth and not pieces of gold or silver'*.**

Kimberlee Musser – New York State DOH
Angela Starks – CDC
Susanne Crowe – Florida DOH
Varsha Hampole & Pennan Barry – California DPH

Acknowledgments!


Sawtooth – 12,304 ft / 3,750 m


Matterhorn 14,691 ft / 4,478 m



Fighting TB
Fighting poverty
Standing up for peace

Thank you!

max@usf.edu