TB 101 – the Laboratory Connection

Global TB Institute Rutgers

College of Public Health
Max Salfinger, MD - FAAM – FIDSA - ATSF | July 29,2021

USF Health
UNIVERSITY of SOUTH FLORIDA

The Thinker (1902)
By Auguste Rodin,
French Sculptor
Rodin Museum
Philadelphia

The thinking human being,
Not able to express himself,
Stands at the same level as
Those who cannot think.

[Pericles, 495- 429 B.C.]

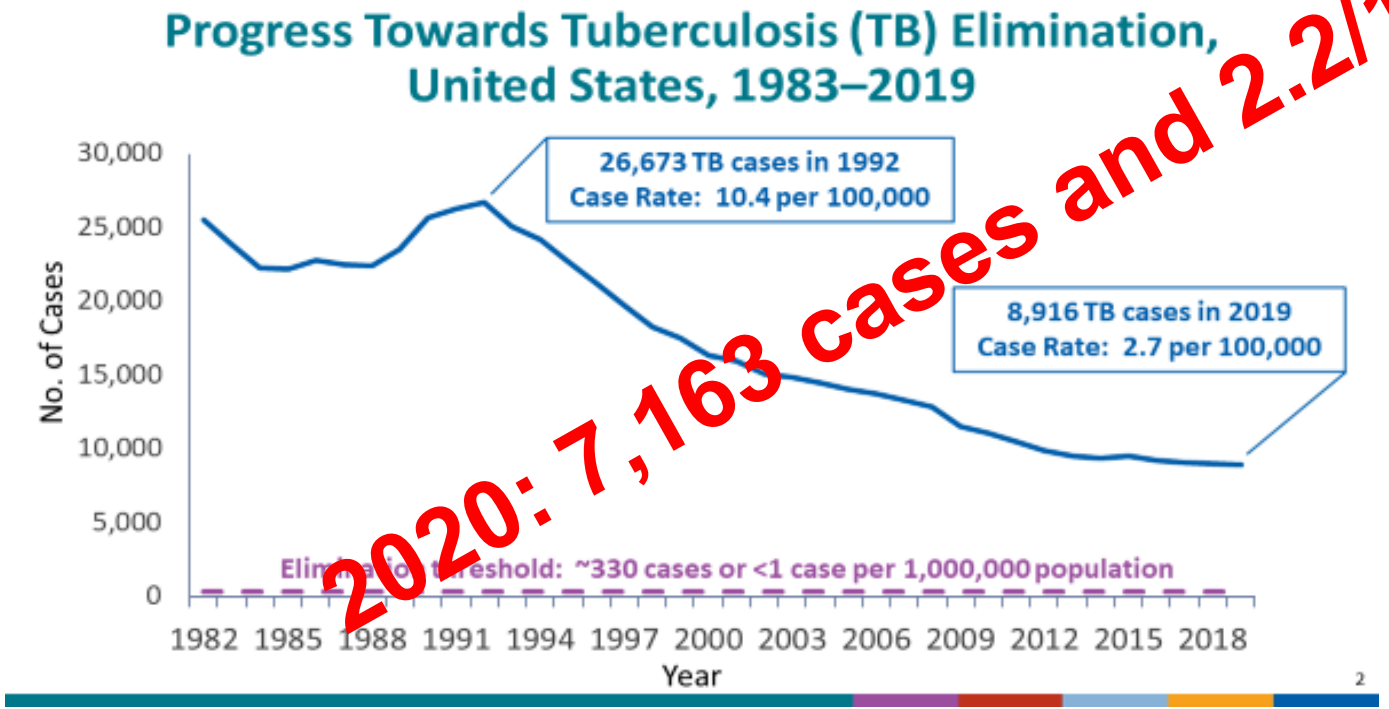
Topics

- Introduction
- Direct Detection (NAAT)
- Decision to Discontinue Airborne Infection Isolation in Healthcare Settings
- Acid-fast Bacilli (AFB) Smear Microscopy
- Growth Detection
- Identification (including NTM)
- Antimicrobial Susceptibility Testing (AST)
- Systems / Algorithms
- Result Reporting
- Acknowledgments

The journey sets the destination

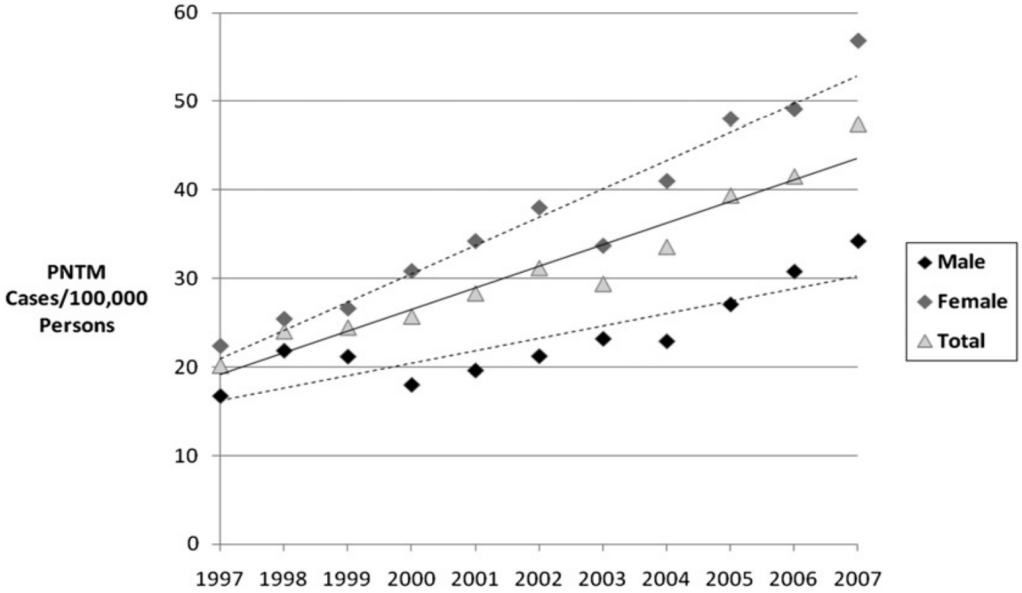
1978-1981	University Hospital, Basel-Switzerland
1981-1992	University of Zurich, Dept. Medical Microbiology, Zurich-Switzerland
1986-1988	Sabbatical – Denver, Colorado
	National Jewish Health, University Hospital, Webb-Waring Lung Institute
1992-2006	Wadsworth Center, Albany, New York
2006-2012	State Public Health Laboratory Director, Tallahassee, Florida
2012-2018	Advanced Diagnostic Laboratories, National Jewish Health, Denver, CO
2018 –	**University of South Florida, College of Public Health, Tampa, FL**
	DrPH Program - Public Health and Clinical Laboratory Science and Practice

Tuberculosis – US, 1993-2019 (CDC)



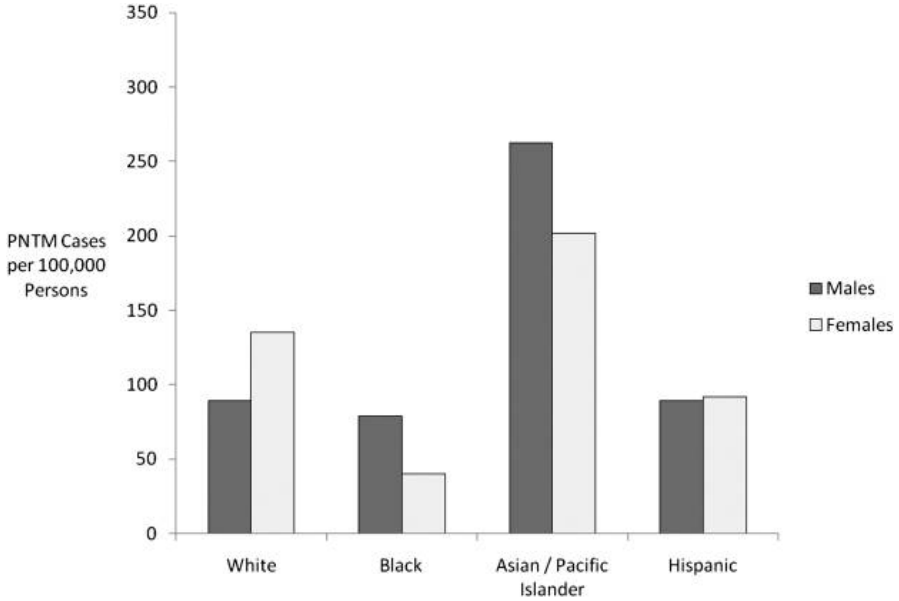
2020: 7,163 cases and 2.2/100k

Pulmonary NTM cases – age 65 and older 1997-2007



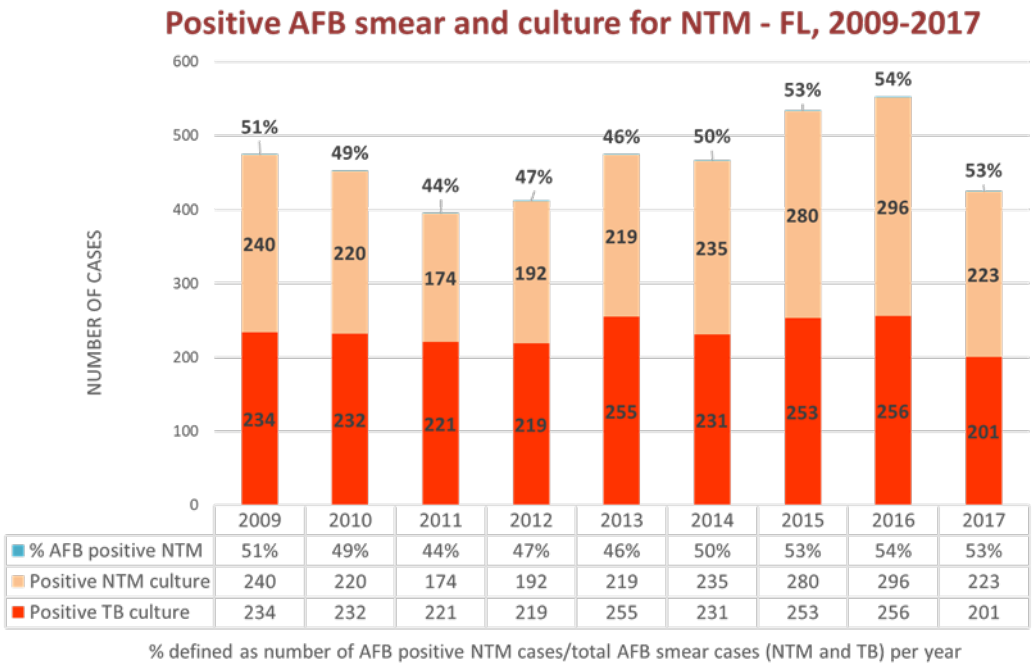
Annual prevalence of pulmonary nontuberculous mycobacteria cases among a sample of U.S. Medicare Part B enrollees by sex from 1997 to 2007. PNTM ¼ pulmonary nontuberculous mycobacteria. PNTM = pulmonary nontuberculous mycobacteria. Adjemian et al 2012

Pulmonary NTM cases – age 65 and older 1997-2007



Period prevalence of pulmonary nontuberculous mycobacteria cases among a sample of **U**.S. Medicare Part B enrollees aged 65 and older from 1997 to 2007 by sex and race/ethnicity. PNTM = pulmonary nontuberculous mycobacteria. Adjemian et al 2012

FL: AFB positive respiratory specimens NTM > TB



	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

Positive AFB smear and culture for mycobacteria, FL, 2009-2017. (FDOH, Bureau of Public Health Laboratories, 2017).

Laboratory's charge

To provide the clinician with accurate results in a timely fashion

Toolbox 1

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

- NALC-NaOH versus Oxalic acid (CF w/history of *Pseudomonas aeruginosa*)
- AFB microscopy
- Solid (NTM plate) & broth-based media
- **NAAT-D** (TB complex, NTM - mostly MAC)
- **NAAT-R** (RIF, INH and more)
- **Direct AST**

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

Ideally, 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
- Minimal Inhibitory Concentration (MIC)

✓**Population management/genotyping**
- RFLP-IS*6110*, Spoligotyping and MIRU –
- whole genome sequencing
- standardization through contracted PHL-Michigan

Quality specimen

Quality testing

requires

quality specimen

[5 to 10 ml sputum]

Acceptable specimens and rejection criteria

Most specimens sent to the laboratory that are acceptable for routine bacterial culture are also acceptable for processing for AFB; however, **every laboratory should develop specific criteria for acceptance and rejection to provide methods for the optimal isolation of *Mycobacterium* spp.** Most specimens will be obtained from the respiratory tract, especially expectorated and induced sputum (the optimal volume is 5 to 10 ml), bronchial aspirates, and bronchoalveolar lavage fluids. Furthermore, the 2017 ATS/IDSA/CDC TB diagnosis guidelines recommend that **post bronchoscopy sputum specimens be collected from all adults with suspected pulmonary TB who undergo bronchoscopy.**

Quality specimen

Sputum, expectorated or induced:

Collection: **Instruct patients** on the proper method of sputum collection

- the material brought up from the lungs after a productive cough what is desired, and not nasopharyngeal discharge and saliva

- **5 - 10 mL sputum** collected in a sterile container.
- Difficulty in producing sputum
  - sputum induction by inhalation of an aerosol of sterile hypertonic saline (3%) or sterile water produced by a nebulizer that causes coughing. **Label as INDUCED**
- Perform in areas with adequate environmental controls under supervision.
- 3 consecutive specimens in 8- to 24-hour intervals, with at least **one being an early morning specimen**.
- Sputum specimens should not be pooled.

Quality specimen

CSF:

- Collection: At least 5 mL of CSF should be aseptically collected.
- Minimum volume required: 2 to 3 mL; optimal volume is 10 mL.
- A separate sample should be collected for chemistry and hematology.

Gastric Lavage:

- Collection: **Specimens should be collected in early morning before patients eat and while they are still in bed.** The lavage should be performed with 25 to 50 mL of chilled, sterile, distilled water. Recovered sample should be placed in a leak-proof, sterile container (e.g., 50-mL conical tube).
- Transport: Gastric wash or lavage material should be submitted in a sterile leak-proof container, such as a sterile 50-mL conical tube or sterile urine collection container.
- Transport time and temperature: Specimens should be transported at room temperature as soon as possible.
  - **If transport is delayed for more than one four hour, specimens should be neutralized with 100 mg sodium carbonate within one hour of collection, and transported as soon as possible at room temperature.**

Quality specimen

Abscess:

- Tissue (at least 1 g, if possible) or fluid is preferred. Tissue should not be frozen or preserved.
- A swab is strongly discouraged unless it is the only specimen available. Swabs should be submitted in 2 to 3 mL sterile saline. Swabs submitted in transport medium or a commercial swab transport device are unacceptable.

Blood:

- Collection: Manufacturer's instructions for automated blood culture systems should be followed.
- **Alternatively**, 10 mL whole blood should be collected aseptically in a yellow-top collector tube containing SPS, or green-top collector tube containing heparin.
- Blood must not be collected in a red-top tube, EDTA (purple top), or ACD (yellow top).
- Minimum volume is 5 mL for adults; 1 mL for children.

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TB NAAT

- FDA approved for respiratory specimens
  - Smear-positive (December 1995)
  - Smear-negative (September 1999)
- **MMWR, January 16, 2009 [Universal]**

In July 2013, the FDA granted Market Authorization to a cartridge-based assay. This NAAT can simultaneously identify *Mycobacterium tuberculosis* complex (TBC) and genetic mutations associated with resistance to rifampin from raw sputum and concentrated sputum sediments.

TB 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

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 (or SR). If volume is insufficient, request an additional sputum specimen for AFB smear and culture.
- Report results from a cartridge-based 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 Sept 2013

TB NAAT comparison

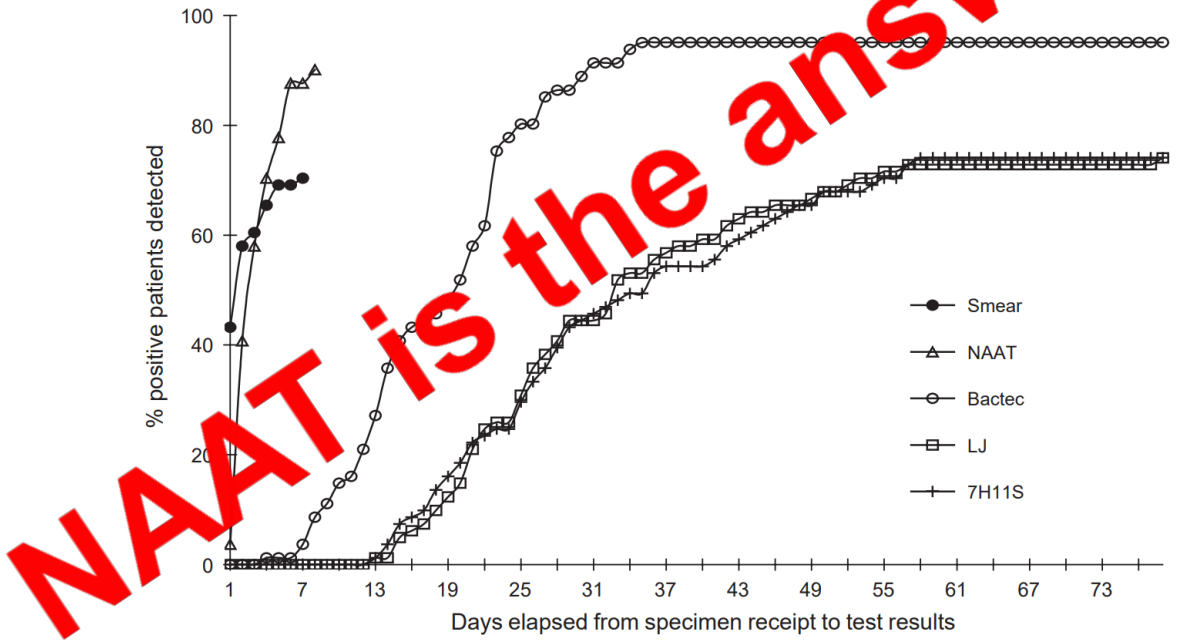
		AFB Smear +	Smear -
MTD*		97%	76%
Laboratory Developed Test**		99.6%	75.4%
Xpert***		100%	71.7%
Xpert – Ultra****	-&+/+	90%	63%
Xpert ****	-&+/+	77%	46%

* Greco et al Thorax 61:783-790(2006)
** Halse et al JCM 48:1182-1188(2010)
*** Helb et al JCM 48:229-237(2010)
**** Dorman et al Lancet ID18:76-84(2018)

Ultra: CE-marked – Not FDA approved

1 NAAT vs. 3 AFB Smear & Culture

(Moore/Guzman/Mikhail – DMID 2015)



NAAT is the answer!

Fig. 2. TAT to report TB-positive patients. The elapsed time from specimen receipt to assay results was evaluated under normal working conditions in the laboratory. The results are expressed as the percentage of TB-positive patients that were reported as positive by the day indicated. The results include all 3 specimens for AFB smear and culture techniques and the first specimen for NAAT.

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Decision to discontinue airborne infection isolation in healthcare settings

NTCA/APHL Consensus Statement on the Use of Cepheid Xpert MTB/RIF Assay in Making Decisions to Discontinue Airborne Infection Isolation in Healthcare Settings

- It is important to note that the process described herein is not to be used alone to rule out TB; Xpert negative or acid-fast bacilli (AFB) smear-negative sputum may contain viable organisms and represent infectious tuberculosis.
- Furthermore, NAA testing should not be used to monitor response to treatment or to release a newly confirmed TB patient from AII.

April 2016

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 savings ($$$)

- 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**.

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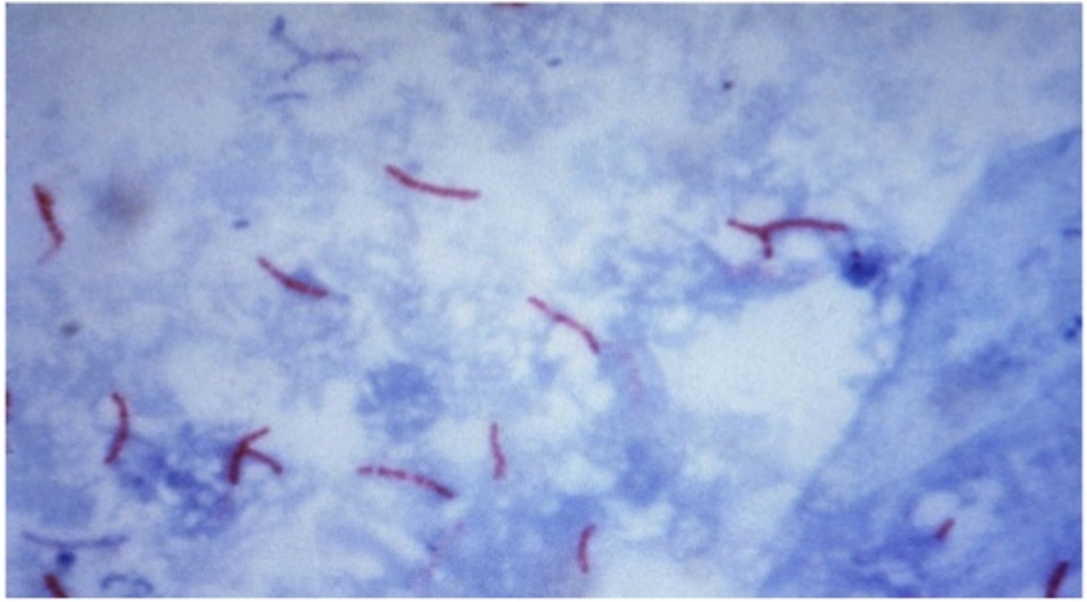
Mycobacterium tuberculosis - Ziehl-Neelsen Staining




AURAMINE-RHODAMINE STAIN
DR.T.V.RAO MD
36

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.

Clinical and Laboratory Standards Institute (CLSI) M48 2nd ed. (2018)

Table 5. Proposed Method for Reporting the Average AFB Number Observed in Patient Specimens at Various Magnifications Using Fluorescence and Ziehl-Neelsen Microscopy

Fluorescence Microscopy		Ziehl-Neelsen	
250×*	450×*	1000×	Report as:
0/smear	0/smear	0/smear	No AFB seen
1–2/30 fields†	1–2/70 fields†	1–2/300 fields†	Report exact count Recommend submission of repeat specimen†
1–9/10 fields	2–18/50 fields	1–9/100 fields	1+
1–9/field	4–36/10 fields	1–9/10 fields	2+
10–90/field	4–36/field	1–9/field	3+
>90/field	>36/field	>9/field	4+

* If the laboratory is using a fluorescent microscope with 200× or 400× magnification, the AFB number observed per number of fields must be revised to meet the five reporting categories.

† Only 1 to 2 AFB per 300 fields is not considered positive but indicates that another specimen should be requested and another smear made from the new specimen.

Abbreviation: AFB, acid-fast bacilli.

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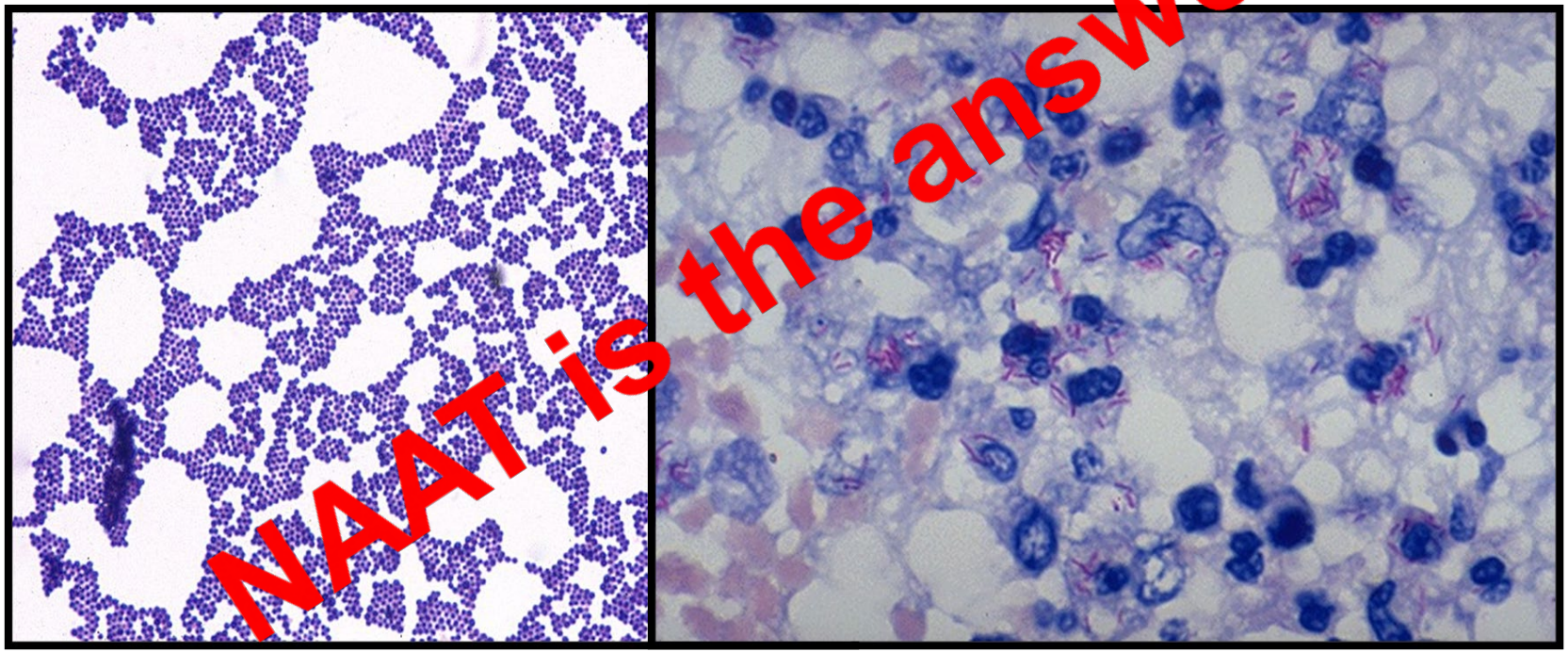
Demanding instant results!



20 Min

20 Hours

Processing sputum samples

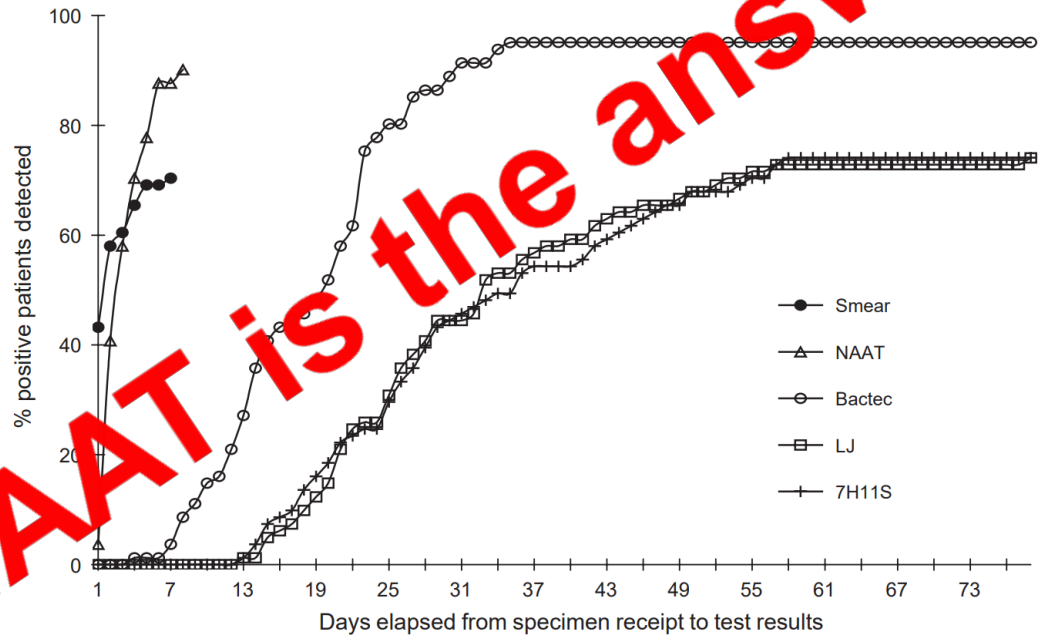
- Procedures kill all but **10-20%** of the mycobacteria
- Contamination

  2-5% of sputum specimens on Loewenstein-Jensen medium (LJ)

1 NAAT vs. 3 AFB smear & culture

(Moore/Guzman/Mikhail – DMID 20[illegible]5

NAAT is the answer!



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Mycobacterium species

194 Species and **13** Subspecies in genus *Mycobacterium* as of July 28, 2021

M. tuberculosis complex

***M. tuberculosis; M. bovis; M. bovis* BCG;**
***M. africanum*;**

M. caprae; M. microti; M. canettii;
M. pinnipedii; M. mungi; M. orygis

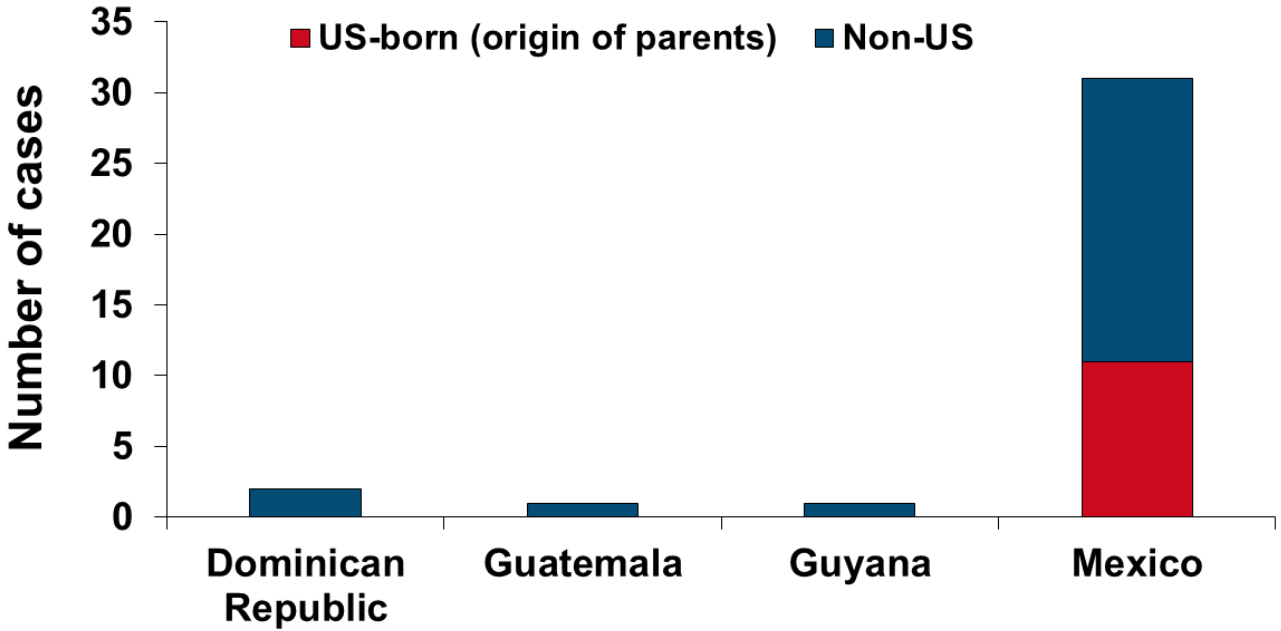
Mycobacterium tuberculosis complex

	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

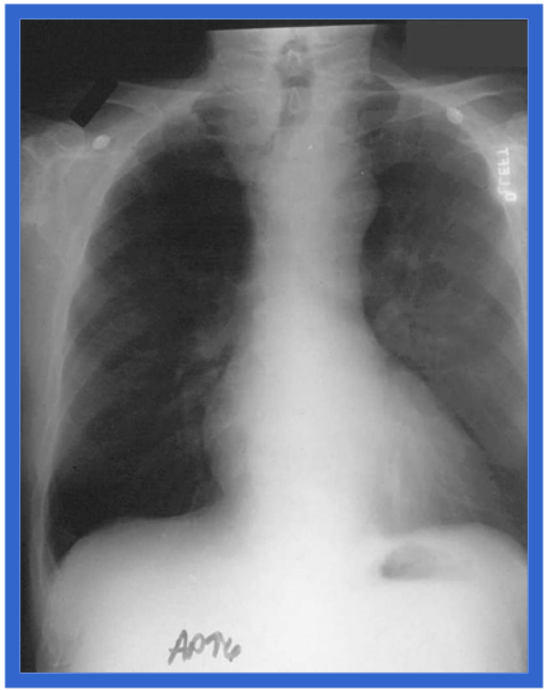
Rapid and Simple Approach for Identification of *Mycobacterium tuberculosis* Complex Isolates by PCR-Based Genomic Deletion Analysis - Parsons et al JCM 40:2339 -2345 (2002)

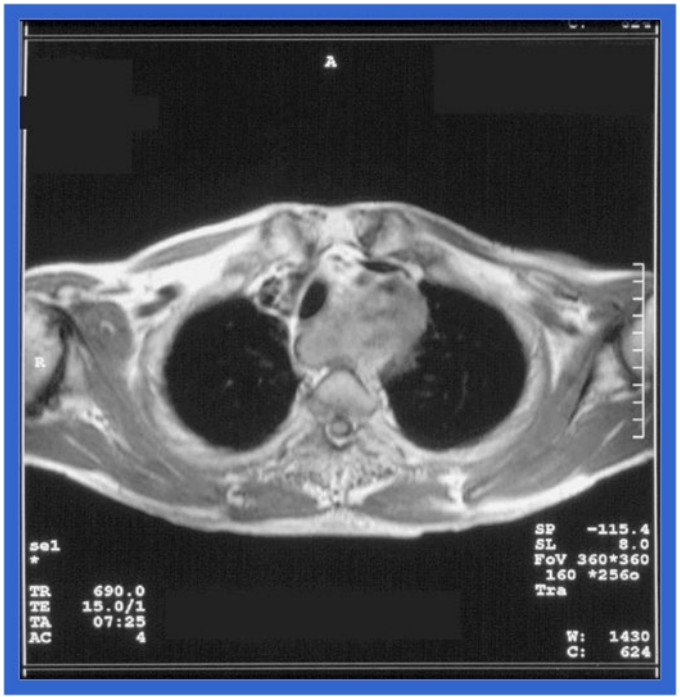
Human TB (N=35) by *Mycobacterium bovis* New York City 2001 – 2004



Winters et al 2005 MMWR 54:605-608

Bladder cancer with *M. bovis* BCG treatment





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

Mycobacterial species in pulmonary NTM

Four integrated health care delivery systems*, 1991-2007

• ***M. avium* complex**	1,495	(80.1%)
• ***M. chelonae/abscessus***	225	(12.1%)
• *M. fortuitum*	106	(5.6%)
• *M. kansasii*	102	(5.5%)
• *M. simiae*	53	(2.8%)
• *M. xenopi*	33	(1.7%)

*KP Southern California, KP Southern Colorado, Group Health, Geisinger

Am J Respir Crit Care Med 2010 Prevots et al.

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Reference antimicrobial susceptibility testing

Agar Proportion Method [CLSI M24]:

- % resistant colonies
- Recognition of mixed cultures
- Up to 3 weeks' incubation
- Direct AST (AFB+ smears)

- **Broth-based methods [WHO]:**
  - Susceptible vs. resistant
  - Shorter TAT
  - Walk-away system
  - Strains with elevated MICs under-recognized

CLSI M24 3rd edition MGIT & VersaTrek

Appendix C. Drugs Available for *Mycobacterium tuberculosis* complex Susceptibility Testing Using Regulatory Organization–Cleared or –Approved Commercial Short-Incubation Liquid Media Systems* and Their Equivalence in the Agar Proportion Method

Antituberculous Agent	System and Concentration, µg/mL		
	Fluorescence-based Detection System	Pressure-based Detection System	Agar Proportion Middlebrook 7H10 Equivalent
Isoniazid	0.1	0.1	0.2
Isoniazid	0.4	0.4	1.0
Rifampin	1.0	1.0	1.0
Ethambutol hydrochloride	5.0	5.0	5.0
Ethambutol hydrochloride	7.5†	8.0	10.0
Pyrazinamide	100	300	–‡
Streptomycin	1.0	–†	2.0
Streptomycin	4.0	–†	10.0

* Cleared for use as of this standard's completion.
† Not available for sale in the United States.
‡ Not available or not recommended.

WHO Technical Report, 2021*

Table 1. Critical concentrations for INH and the rifamycins.

Drug	LJ	7H10	7H11	MGIT
Isoniazid	0.2	0.2	0.2	0.1
Rifampicin[a]	40	0.5	1.0	0.5
Rifabutin[b]	–	–	–	–
Rifapentine[c]	–	–	–	–

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Changes to the previous version of the table are highlighted in red.[8]

*Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)

Molecular detection of drug resistance

Drug	Gene	Sens.	Spec.
RIF	*rpoB*	97.1%	97.4%
INH	*katG, inhA*	86.0%	99.1%
EMB	*embB*	78.8%	94.3%
PZA	*pncA*	86.0%	95.9%
F-quinolones	*gyrA*	79.0%	99.6%

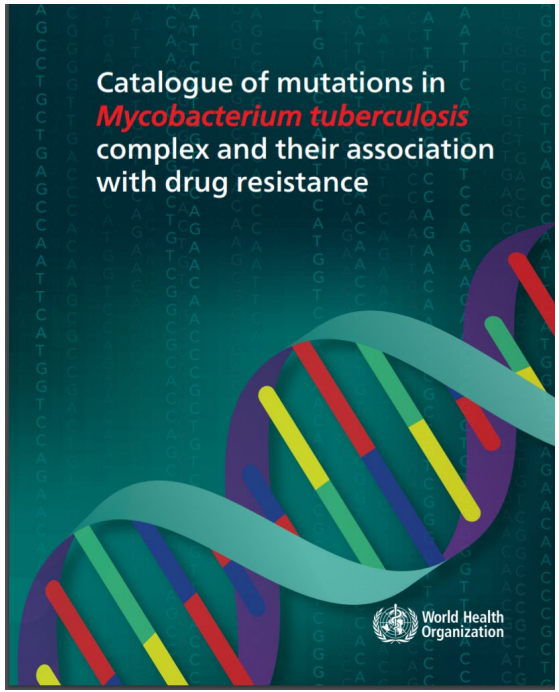
Curry Center: Drug-resistant tuberculosis – A survival guide for clinicians, 3rd ed. 2016

Molecular testing - limitations

- Potential to identify mutations that do not confer phenotypic resistance
- Not all genetic loci associated with resistance are known; therefore,

‘no mutation detected’ does not rule out resistance

WHO Catalogue of mutations in MTBC and their association with drug resistance, 2021



15 antimicrobials
38,000 isolates
>17,000 mutations

https://www.who.int/publications/i/item/9789240028173

Molecular detection of drug resistance

- Acid-fast smear-positive specimen
- Some of the specimen sediment is available for sending to reference lab (State Public Health Lab)
- Drug resistance is suspected, or
- A susceptible population has been exposed, or
- The culture is mixed or non-viable, so regular antimicrobial susceptibility testing can't be done
- CDC also has **Molecular Detection of Drug Resistance (MDDR) program**: tests for mutations associated with resistance to additional drugs—ethambutol, pyrazinamide

Universal

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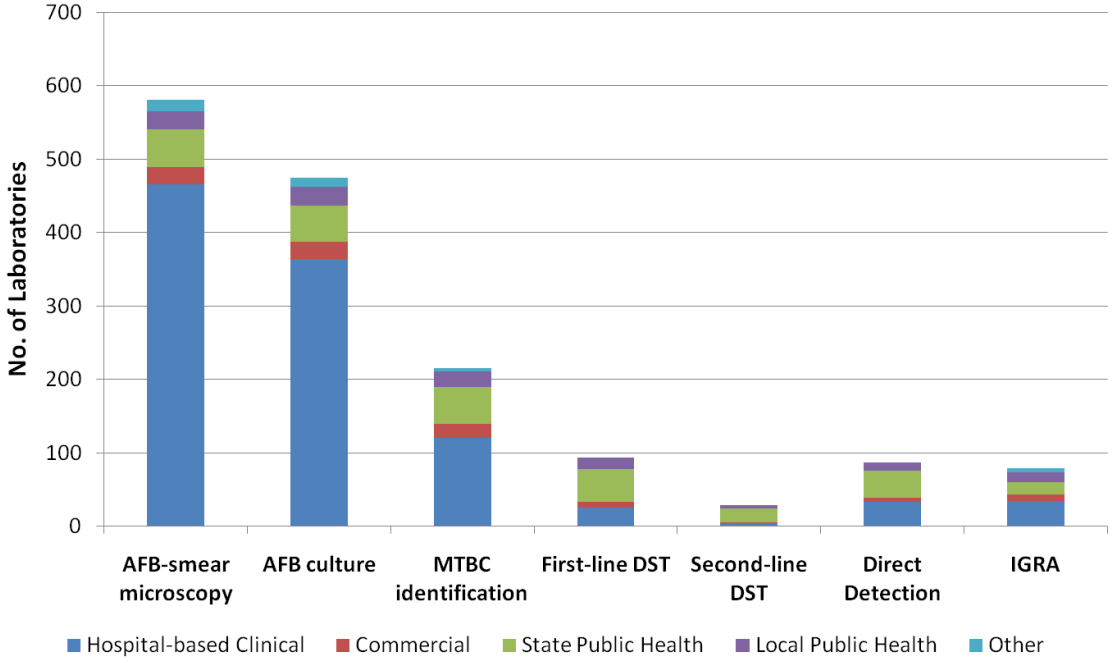
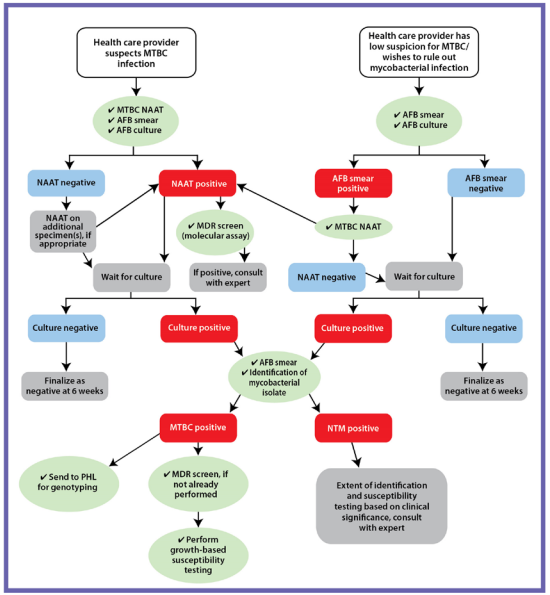
In-house AFB service performed - APHL/CDC Survey 2011



Practice guidelines for clinical microbiology laboratories: Mycobacteria, 2018



TB WGS Timeline

2020/2021

- Reduced cost Nextseq
- Reduced TAT
- **3000 TB genomes**
- Direct specimen NGS
- External Pipeline

2019

- >1-year reduced DST Comparison to MIC data
- NIH R21 MinION

2015

- Validation, Extraction Development
- Guidance created
- RFA Establishment of MTBC WGS Reference Centers
- NIH R01 TB WGS Sputum

2014

- **First TB WGS in NYS**
- Analytical Pipeline Construction

2018

- Updated reporting
- New reduced DST algorithm
- **2000 TB genomes**

2016

- First clinical WGS report
- Universal WGS in NYS

2017

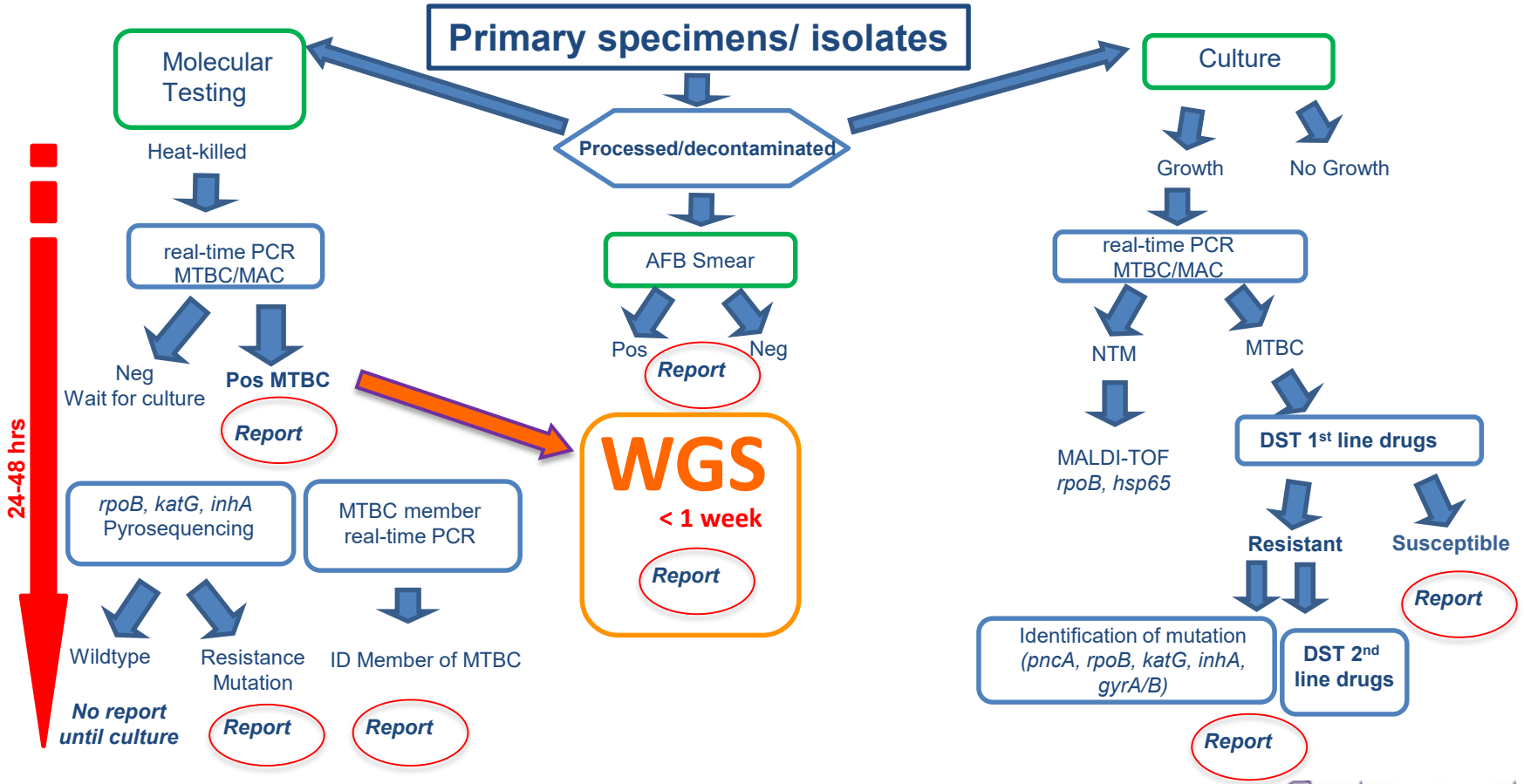
- ReSeqTB contributions
- Major TB WGS Improvements
- **1000 TB genomes**

2013

- 2013 WC Public Health Genomics Center internal funding opportunity pilot

NEW YORK STATE | Department of Health | Wadsworth Center

Testing Algorithm



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Laboratory information management system

- Electronic laboratory testing ordering
- Electronic laboratory resulting
- **Billing**
- Algorithm
- Inventory management
- Sample centric versus patient centric
- Integrate instruments
- Audit trails
- Data storage
- Sample management

LIMS/LIS - vendors

Mycobacterium avium identification - sequencing

IDENTIFICATION BY SEQUENCING	*Mycobacterium avium*

COMMENT:	By partial 16S rRNA gene sequencing , this isolate matches the *M.avium* type strain 100%.

Guidelines:

The following algorithm can be applied for partial or complete 16S rRNA gene sequences, per in house validation:

1.100% identity for Genus and species identification; report "[*Genus and species*]".

2. 99.0% to 99.9% identity for *Genus* identification; consider reporting "[*Genus*], most closely related to [*species*]".

3. 95%-98.9% cannot be definitively identified by 16S rRNA gene sequencing; consider reporting "Unable to identify by 16S rRNA gene sequencing, most closely related to [*Genus*]".

For *erm* (41) and *erm* (39) gene sequencing, the following can be applied:

1. Subspecies of *M. abscessus* complex can be determined by *erm* (41) gene sequencing

2. *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* can be determined by both *rpoB* and *erm* (41) gene sequencing.

3. *M. fortuitum* group that give indeterminate results with *rpo* β gene sequencing, can be speciated by *erm* (39) gene sequencing

For *rpo* β gene sequencing of rapidly growing mycobacteria, the following can be applied:

1.For 98.3%-100% identity consider reporting "[Genus and species]".

2.For 97.0% -98.2% identity consider reporting "[*Genus*], most closely related to [*species*]".

3.For 83.9-96.9% consider reporting "Unable to identify by *rpoB* gene sequencing, most closely related to *Mycobacterium sp.* ".

REFERENCES

1. Fast Microseq 500 16S rDNA Bacterial Identification Kits, Manufacturer's Protocol, Applied Bio-systems, USA.
2. CLSI, MM18-A, 2008. CLSI MM18A-2, 2018.
3. Utility of *erm* (41)Brown- Elliott et.al. April 2015 Vol. 53 No. 4; *Journal of Clinical Microbiology*
4. *rpoB* -based identification- Adekambi et.al. Dec 2003. Vol. 41 No. 12; *Journal of Clinical Microbiology*
5. Intrinsic Macrolide resistance in RGM- Nash et.al. Oct. 2006. Vol. 50, No. 10; *Antimicrobial Agents and Chemotherapy*

Identification by DNA sequencing

This test was developed and its performance characteristics determined by UTHSCT. It has not been cleared or approved by FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or research work.

The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. Testing of a secondary gene or full length 16S rRNA may be required to distinguish between subspecies or closely related species.

Richard J. Wallace Jr., M.D.

Mycobacteria/Nocardia Laboratory

The University of Texas Health Science Center at Tyler,
11937 US Hwy 271, Tyler, Texas 75708
Phone: (903) 877-7685
FAX: (903) 877-7652

UTHealth North Campus Tyler

Direct molecular drug resistance - pyrosequencing

Clinical Mycobacteriology Laboratory
Phone: (518) 474-4158 Fax: (518) 408-2264

Testing performed at CLIA# 33D2005937

Specimen Id: IDR2000254083

Specimen Type: Sputum

Concentrated Smear(Ziehl - Neelsen/1,000 X)		
(10/26/20):	**Numerous (>9 acid-fast bacilli per field)**	10/26/2020
Direct Molecular Detection - Real-time PCR		
Mycobacterium tuberculosis complex DNA by real-time PCR*:	**DETECTED**	10/27/2020
Mycobacterium avium complex DNA by real-time PCR*:	**Not Detected**	10/27/2020
Molecular Identification - Real-time PCR		
Mycobacterium tuberculosis complex species DNA identified*:	**Mycobacterium tuberculosis**	10/28/2020
Direct Molecular Drug Susceptibility Detection- Pyrosequencing		
Rifampin (rpoB)*:	**Mutation absent suggests no rifampin resistance. Whole genome sequencing must be performed for final susceptibility result.**	10/30/2020
Isoniazid (katG)*:	**Mutation absent suggests no isoniazid resistance. Whole genome sequencing must be performed for final susceptibility result.**	10/30/2020
Isoniazid (inhA)*:	**Mutation absent suggests no isoniazid resistance. Whole genome sequencing must be performed for final susceptibility result.**	10/30/2020
Culture		
(11/02/20):	**acid-fast bacillus was isolated**	11/3/2020

TB NAAT

AFB Mycobacterium TB Complex NAA Order: 1609216916 - Reflex for Order 1609030707

Status: **Final result** Visible to patient: **No (not released)** Next appt: **None**

Specimen Information: SPUTUM EXPECTORATED

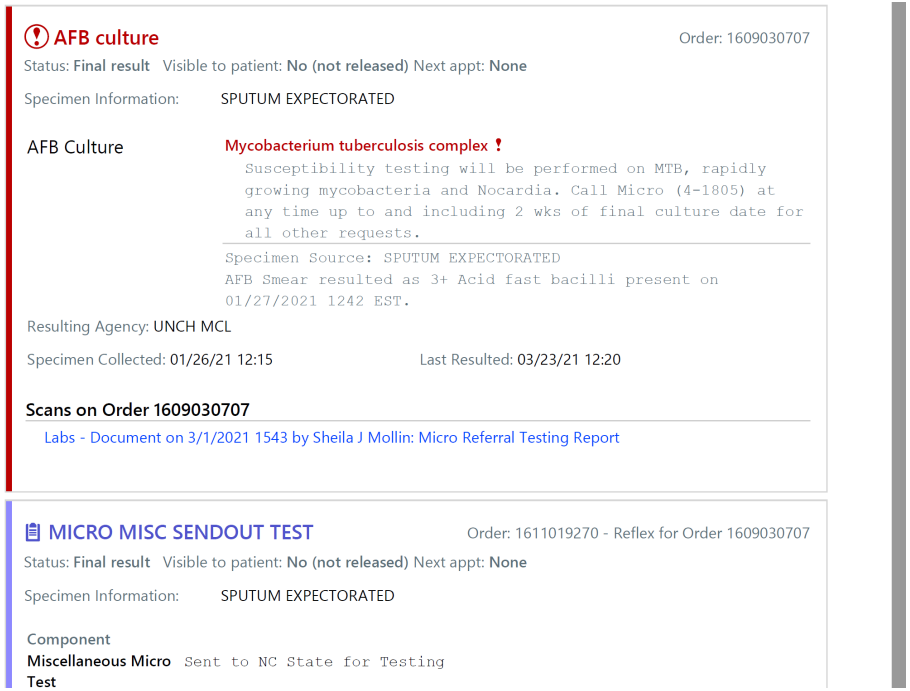
	Ref Range & Units	1/26/21 1215
MTB NAAT	Negative	**Positive** !
Rifampin Resistance	Not Detected	Not Detected
Resulting Agency		UNCH MCLENDON CLINICAL LABORATORIES

Narrative Performed by: UNCH MCL

Specimen Source: SPUTUM EXPECTORATED
-
This test was performed using the FDA-cleared Cepheid Xpert MTB/RIF assay, which targets the rpoB gene of Mycobacterium tuberculosis complex (MTBC) members. Performance characteristics have been established and verified by the Clinical Molecular Microbiology Laboratory, UNC Medical Center. The sensitivity of MTBC detection for smear-positive respiratory specimens is 98-100% while the sensitivity of MTBC detection for a single smear-negative respiratory specimen is 70-72%. Specificity of MTBC detection for respiratory specimens is 99-100%. The rifampin resistance screen is 98% accurate based on in-house data and provides only preliminary results. Confirmation of rifampin results requires phenotypic susceptibility testing performed on an MTBC isolate. Clinical and epidemiologic correlation and close monitoring of culture results are strongly recommended. For additional information about this assay see: N Engl J Med. 2010 Sep 9; 363(11):1005-15.

Specimen Collected: 01/26/21 12:15 Last Resulted: 01/27/21 15:23

Referral TB AST to NC State Laboratory



AFB culture Order: 1609030707

Status: **Final result** Visible to patient: **No (not released)** Next appt: **None**

Specimen Information: SPUTUM EXPECTORATED

AFB Culture — **Mycobacterium tuberculosis complex !**

Susceptibility testing will be performed on MTB, rapidly growing mycobacteria and Nocardia. Call Micro (4-1805) at any time up to and including 2 wks of final culture date for all other requests.

Specimen Source: SPUTUM EXPECTORATED
AFB Smear resulted as 3+ Acid fast bacilli present on 01/27/2021 1242 EST.

Resulting Agency: UNCH MCL

Specimen Collected: 01/26/21 12:15 Last Resulted: 03/23/21 12:20

Scans on Order 1609030707

Labs - Document on 3/1/2021 1543 by Sheila J Mollin: Micro Referral Testing Report

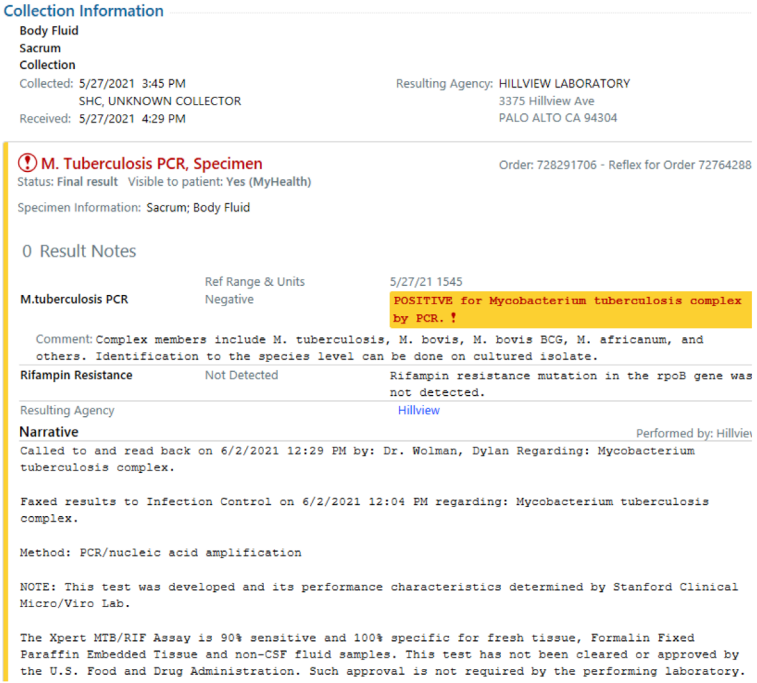
MICRO MISC SENDOUT TEST Order: 1611019270 - Reflex for Order 1609030707

Status: **Final result** Visible to patient: **No (not released)** Next appt: **None**

Specimen Information: SPUTUM EXPECTORATED

Component
Miscellaneous Micro Test Sent to NC State for Testing

Xpert off-label use



Collection Information

Body Fluid
Sacrum
Collection
Collected: 5/27/2021 3:45 PM
SHC, UNKNOWN COLLECTOR
Received: 5/27/2021 4:29 PM

Resulting Agency: HILLVIEW LABORATORY
3375 Hillview Ave
PALO ALTO CA 94304

M. Tuberculosis PCR, Specimen
Order: 728291706 - Reflex for Order 72764288
Status: **Final result** Visible to patient: **Yes (MyHealth)**

Specimen Information: Sacrum; Body Fluid

0 Result Notes

	Ref Range & Units	5/27/21 1545
M.tuberculosis PCR	Negative	**POSITIVE for Mycobacterium tuberculosis complex by PCR. !**
Comment: Complex members include M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, and others. Identification to the species level can be done on cultured isolate.		
Rifampin Resistance	Not Detected	Rifampin resistance mutation in the rpoB gene was not detected.
Resulting Agency		Hillview

Narrative Performed by: Hillvie

Called to and read back on 6/2/2021 12:29 PM by: Dr. Wolman, Dylan Regarding: Mycobacterium tuberculosis complex.

Faxed results to Infection Control on 6/2/2021 12:04 PM regarding: Mycobacterium tuberculosis complex.

Method: PCR/nucleic acid amplification

NOTE: This test was developed and its performance characteristics determined by Stanford Clinical Micro/Viro Lab.

The Xpert MTB/RIF Assay is 90% sensitive and 100% specific for fresh tissue, Formalin Fixed Paraffin Embedded Tissue and non-CSF fluid samples. This test has not been cleared or approved by the U.S. Food and Drug Administration. Such approval is not required by the performing laboratory.

Rif at 0.5 and PZA MIC

National Jewish Health®

Advanced Diagnostic Laboratories
1400 Jackson Street, Denver, CO 80206
Client Services (p): 800.550.6227 (f): 800.652.9556
ClinRefLabs@njhealth.org njlabs.org
CAP# 2178901 CLIA# 06D0644307

ZZTEST, XXX **Order #: XXX**

Source: Induced Sputum

Collected: 03/09/21 15:06
Received: 03/10/21 17:01

For additional information see Curry International Tuberculosis Center and California Department of Public Health, 2016: "Drug-Resistant Tuberculosis: A Survival Guide for Clinicians, Third Edition" page 46.

	M. tuberculosis	
ANTIBIOTICS	MIC mcg/mL	INTRP
Ethambutol 5.0 mcg/mL - MGIT		S
Isoniazid 0.1 mcg/mL - MGIT		S
Isoniazid 0.4 mcg/mL - MGIT		S
Pyrazinamide	<=50	TS D1
Rifampin 0.5 mcg/mL - MGIT		S
x Compliance Statement		* D1

S=Susceptible I=Intermediate R=Resistant NI=No CLSI interpretive guidelines for this antibiotic/organism combination.
TS=Tentative Interpretation Susceptible TI=Tentative Interpretation Intermediate TR=Tentative Interpretation Resistant

-----DRUG COMMENTS-----

D1 ; Testing was performed using the MGIT 960 methodology. Testing for Pyrazinamide was performed at 50.0, 100.0, 200.0 and 400.0 mcg/mL. A MIC of <=100.0 mcg/mL is considered susceptible.

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This assay is a laboratory developed test used for clinical purposes. It was developed and its performance characteristics determined by Advanced Diagnostic Laboratories at National Jewish Health. It has not been cleared or approved by the U.S.

Corrected/amended report

Culture, AFB

Culture, AFB (Edited)	
Culture, AFB	**Mycobacterium tuberculosis (AA)** Identification confirmed by Michigan Department of Health and Human Services, Lansing, MI This is a corrected result. Previous organism was Mycobacterium tuberculosis complex on 5/27/2021 at 2:53 PM EDT.
Stain, AFB	**(AA)** **Rare acid fast bacilli seen**
Resulting Lab: BLRYO	

AFB notification & disclaimer molecular DR

CRITICAL RESULT PHONED/FAXED at 17:14 on 17 May 21 to [redacted](GGH) by L8PW2 :POSITIVE AFB SMEAR NOTIFIED
Smear report : Positive for acid-fast bacilli 1+

CULTURE

1) Mycobacterium tuberculosis complex
The isolate is predicted to be sensitive to isoniazid, rifampin, pyrazinamide and ethambutol based on molecular testing for resistance associated mutations. Note that the lack of a mutation is insufficient to rule out antibiotic resistance. Phenotypic testing remains the gold standard for the determination of antibiotic resistance. Please correlate these results with phenotypic testing.
For advice regarding interpretation of this report: contact the Microbiologist on call. For advice regarding TB therapy: contact the Respiratory Service or the Infectious Diseases Service.
Testing performed at
National Microbiology Laboratory, Public Health Agency of Canada,
1015 Arlington Street, Winnipeg MB, R3E 3R2

Topics

- Introduction
- Direct Detection (NAAT)
- Decision to Discontinue Airborne Infection Isolation in Healthcare Settings
- Acid-fast Bacilli (AFB) Smear Microscopy
- Growth Detection
- Identification (including NTM)
- Antimicrobial Susceptibility Testing (AST)
- Systems / Algorithms
- Result Reporting
- Acknowledgments

Acknowledgments:

- Beaumont Hospital, Royal Oak, Michigan
- Microbial Diseases Laboratory, Richmond, California
- National Jewish Health, Denver, Colorado
- Public Health Agency of Canada, Winnipeg, Manitoba
- Stanford Healthcare, Palo Alto, California
- The University of Texas Health Science Center at Tyler, Texas
- UNC Health, Chapel Hill, North Carolina
- Wadsworth Center, Albany, New York
- Kim Musser, Wadsworth Center, Albany, New York

Maroon Bells, Colorado

Roseate Spoonbills, Florida

Thank you for the opportunity to be with you today!

max@usf.edu

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