



Wisconsin State
Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON

Diagnostic Testing for Mycobacterium Tuberculosis Complex

Nate Simon
WI TB Laboratory Program Coordinator

WI State Lab of Hygiene – Communicable Disease Division
10/07/2026

Accreditation Statement



Accreditation Statement

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UAN Number: UAN JA0000238-0000-26-089-L99-P.

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Available Credit

- 18.25 ACPE,
- 18.25 *AMA PRA Category 1 Credit™*
- 18.25 ANCC
- 18.25 Attendance
- 18.25 IPCE

Disclosures

- I have no financial interests or relationships to disclose.
- No off-label mentions of any medications; off-label mention of products only as it relates to CLIA-compliant laboratory-developed testing.

Outline

Mycobacterium tuberculosis complex (MTBC) diagnostic testing

- Historical testing
- “Modern” testing
 - Rapid screening
 - MTBC ID from primary specimens
 - Culture ID
 - Susceptibility testing
- Benefits of MTBC molecular diagnostics



What is the primary historical contribution of guinea pigs toward diagnosis of TB infection?

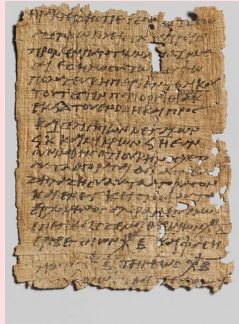
- A. Injection of guinea pigs with patient sputum provided one of the first animal-based methods to confirm TB infection
- B. Guinea pigs were able to identify the smell of TB in patient sputum
- C. Guinea pigs are naturally resistant to TB infection, so provided a negative control organism for identification of the TB bacillus
- D. Guinea pig physiology is identical to humans, so they provided critical information on disease progression

MTBC diagnostics: Ancient bug, antiquated methods

3300BCE

INDIA

First written document describing TB



200AD

**GALEN
(GREECE)**

“Clinical” diagnosis
Fever, sweating,
coughing, bloody
sputum



1860s

**JEAN-
ANTOINE
VILLEMIN**

Animal models
of infection

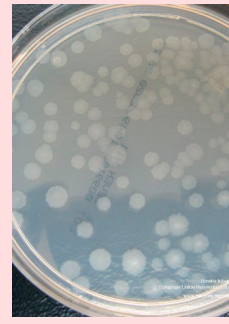
First: rabbits,
Best: guinea pigs



1882

**ROBERT
KOCH**

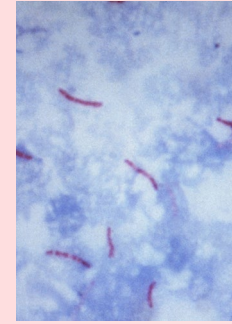
First reported
MTB stain
and culture



1885

**ZIEHL,
NEELSEN,
OTHERS**

Ziehl-Neelsen
AFB stain



1890s

**WILHELM
ROENTGEN**

Chest X-ray



1907

**CHARLES
MANTOUX**

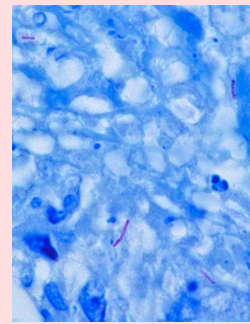
TB skin test



1915

**JOSEPH
KINYOUN**

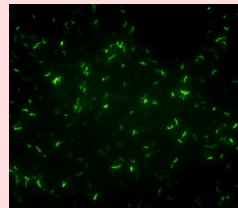
Kinyoun (cold ZN)
stain



MANY YEARS LATER...

1960s

Auramine O
AFB stain



1968

Middlebrook 7H11
media



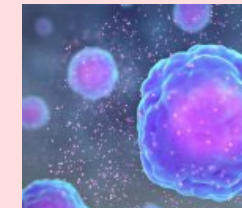
1980s

Automated
culture



1990s

IGRA
NAAT



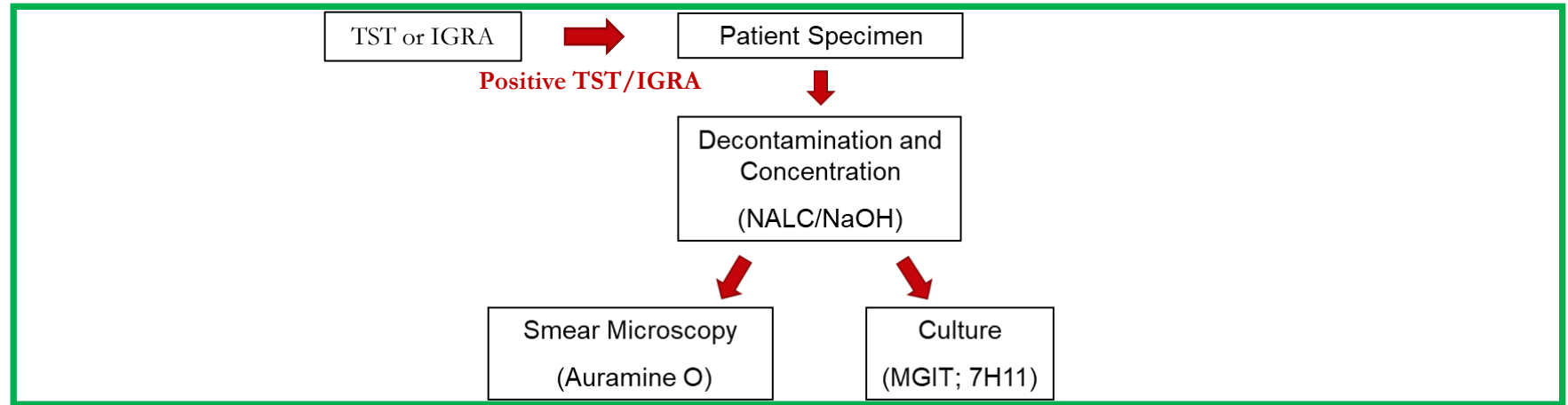
2020s

Next-generation
sequencing

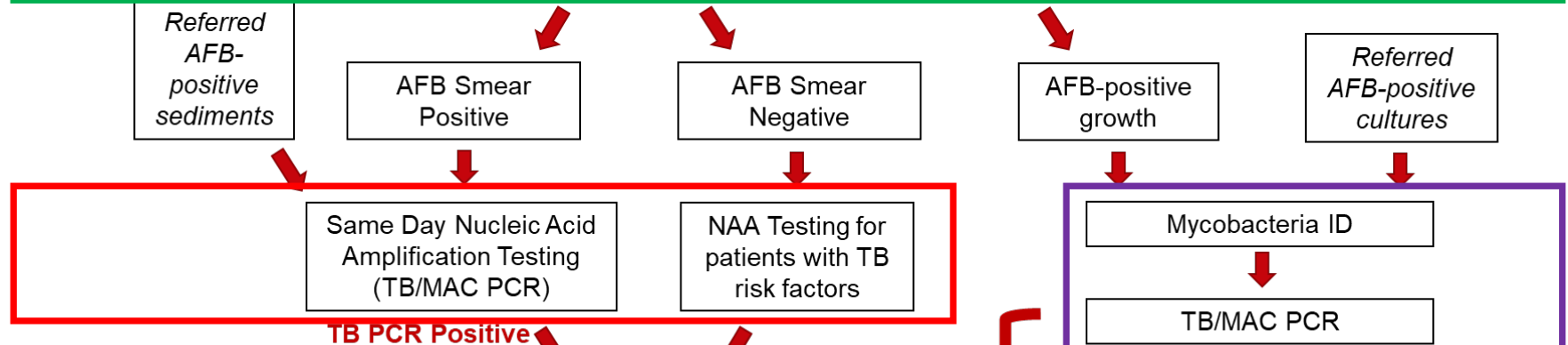


Modern Mycobacteriology

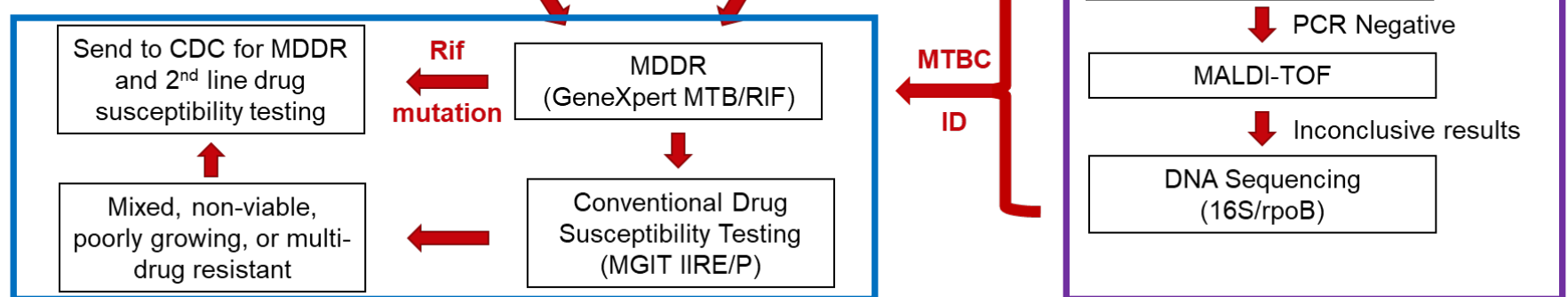
Rapid screen for *potential* MTBC infection



Rapid ID of MTBC from primary specimens



ID of MTBC from positive culture



Access to MTBC drug susceptibility testing

Purpose of classes	Technology class	Included products ^a
Initial tests for TB diagnosis with drug-resistance detection	NEW: Low-complexity automated nucleic acid amplification tests (LC-aNAATs) for detection of TB and resistance to RIF Moderate-complexity automated nucleic acid amplification tests (MC-aNAATs) for detection of TB, and resistance to RIF and INH	Xpert® MTB/RIF Ultra (Cepheid)*
		Truenat® MTB Plus and Truenat MTB-RIF Dx (Molbio) *
		RealTime® MTB and RealTime MTB RIF/INH (Abbott) *
		BD MAX™ MDR-TB (Becton Dickinson)* cobas® MTB and cobas MTB-RIF/INH (Roche)* FluoroType® MTB and FluoroType MTBDR (Bruker-Hain)*
Initial tests for TB diagnosis without drug-resistance detection	NEW: Low-complexity manual nucleic acid amplification tests (LC-mNAATs) for detection of TB	Loopamp™ MTBC Detection Kit (TB-LAMP) (Eiken Chemical) *
	Antigen detection in a lateral flow format (biomarker-based detection; LF-LAM) for detection of TB	Determine™ TB LAM Ag (Alere/Abbott) *
Follow-on tests for detection of TB drug resistance	Low-complexity automated nucleic acid amplification tests (LC-aNAATs) for detection of resistance to INH and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid) *
		GenoType® MTBDRplus v2 (Bruker-Hain)* GenoType MTBDRsl (Bruker-Hain) *
	Line probe assays (LPAs) – for detection of TB drug resistance	Genoscholar™ NTM+MDRTB II (Nipro) *
		Genoscholar PZA-TB II (Nipro) *
	Targeted next-generation sequencing (NGS) for detection of resistance to first-line and second-line anti-TB agents ^b	Deeplex® Myc-TB (GenoScreen/Illumina)
		**UPDATED AmPORE-TB® (Oxford Nanopore Technologies)
		TBseq® (Shengting Medical Technology Co) *
Tests for TB infection	<i>Mycobacterium tuberculosis</i> (Mtb) antigen-based skin tests (TBSTs)	Diaskintest® (Generium)*
		SIILTBCY® (Serum Institute of India)*
		C-TST (Anhui Zhifei Longcom)*
	Interferon-gamma release assays (IGRAs)	QuantiFERON-TB® Gold Plus (QFT-Plus) (QIAGEN)
		T-SPOT®.TB (T-Spot) (Oxford Immunotec)Revvity
		TB-IGRA (Wantai BioPharm) *
		**NEW STANDARD E TB-Feron (ELISA) (SD Biosensor)
		**NEW LIAISON® QFT-PLUS (CLIA) (QIAGEN/ Diasorin)
	Tuberculin skin tests	Tuberculin purified protein derivative (PPD) products

WHO-Endorsed TB Diagnostic Tests

***Not (readily) available in the United States**

- Wide array of point-of-point of care, near-point-of-care, and laboratory tests just not available to majority of US-based laboratories

Rapid screen for *potential* MTBC infection

TB Screening – Has there been TB exposure?

- TB skin test (TST) or Interferon Gamma Release Assay (IGRA)
 - Measure immune response to MTBC antigens
- Advantages:
 - Rapid screening (24-72hr)
 - Specific for MTBC (for the most part)
- Limitations:
 - Requires multiple visits (TST) or laboratory (IGRA)
 - Some non-TB mycobacterial cross-reactivity with TST
 - Not ideal for those with BCG vaccine
 - Require competent immune system



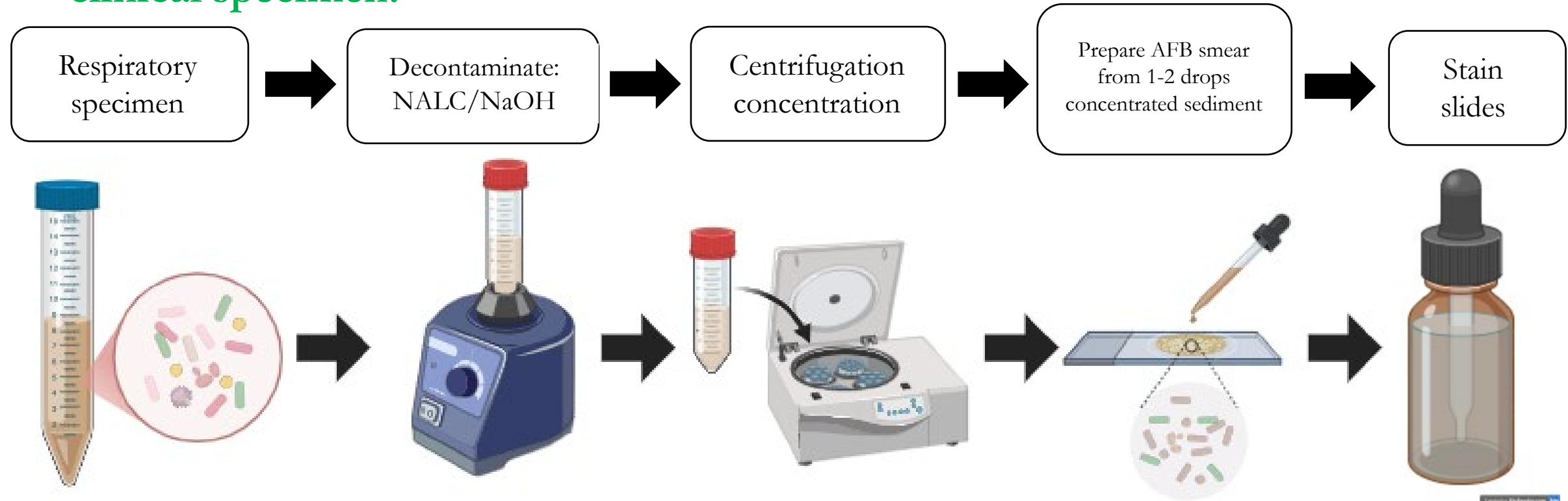
Sanofi



QIAGEN

Rapid screen for *potential* MTBC infection

Acid-fast bacilli (AFB) smear testing – Are AFB present in the primary clinical specimen?



- High-quality specimens are thick/mucopurulent
- 3-5 ml in volume ideal

- Digestion: 0.5% NALC; used to liquefy sputum
- Decontamination: 2% NaOH; used to kill respiratory flora

- 3-50ml of specimen → <1ml concentrated sediment

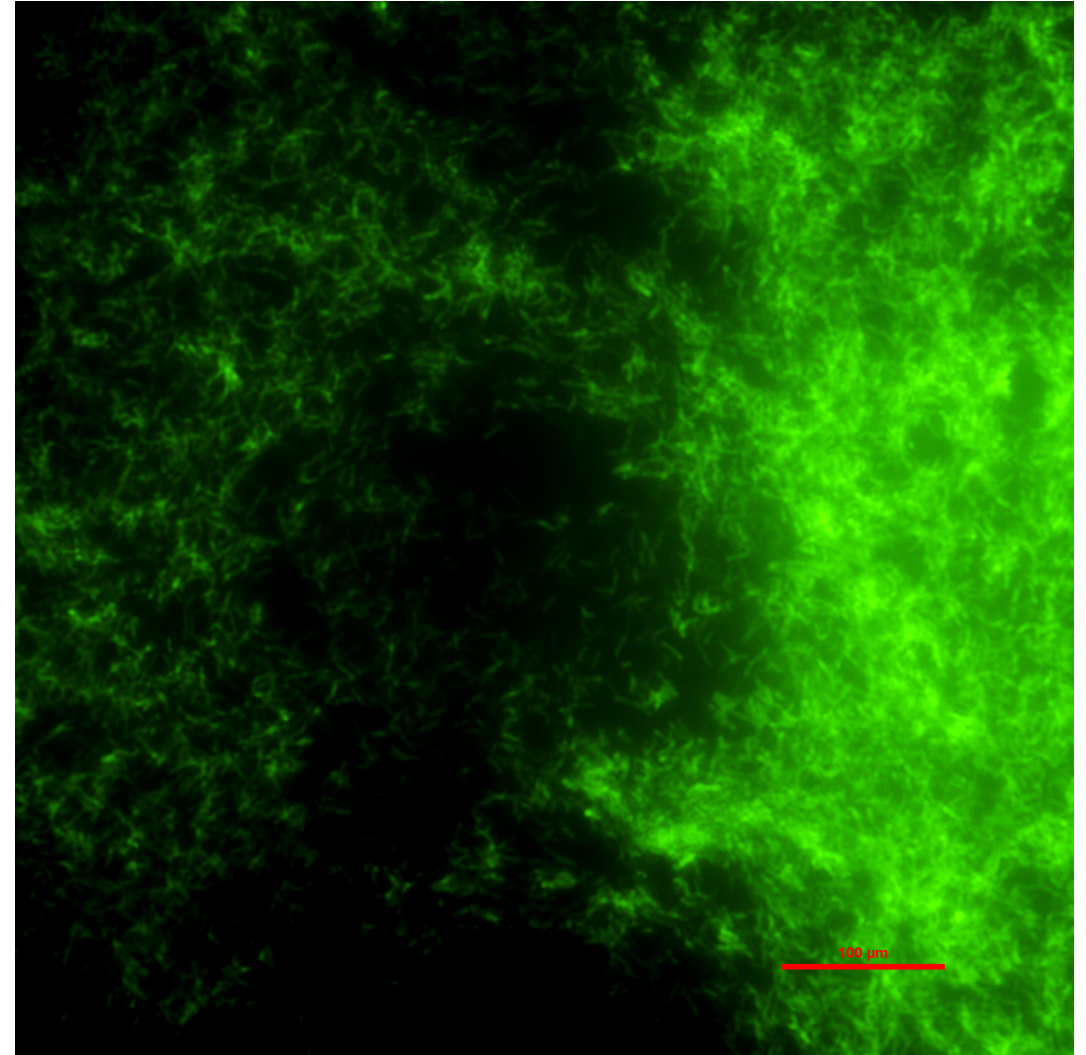
- One slide per patient to prevent cross-contamination

- Auramine O fluorescent stain provides best contrast for easy ID of AFB

Rapid screen for *potential* MTBC infection

AFB smear testing

- Advantages:
 - Rapid and inexpensive screening tool
 - All types of specimens can be examined
- Limitations:
 - Requires specimen processing, staining, and microscopy facilities
 - Limited specificity
 - Limited sensitivity
 - Does not indicate viability



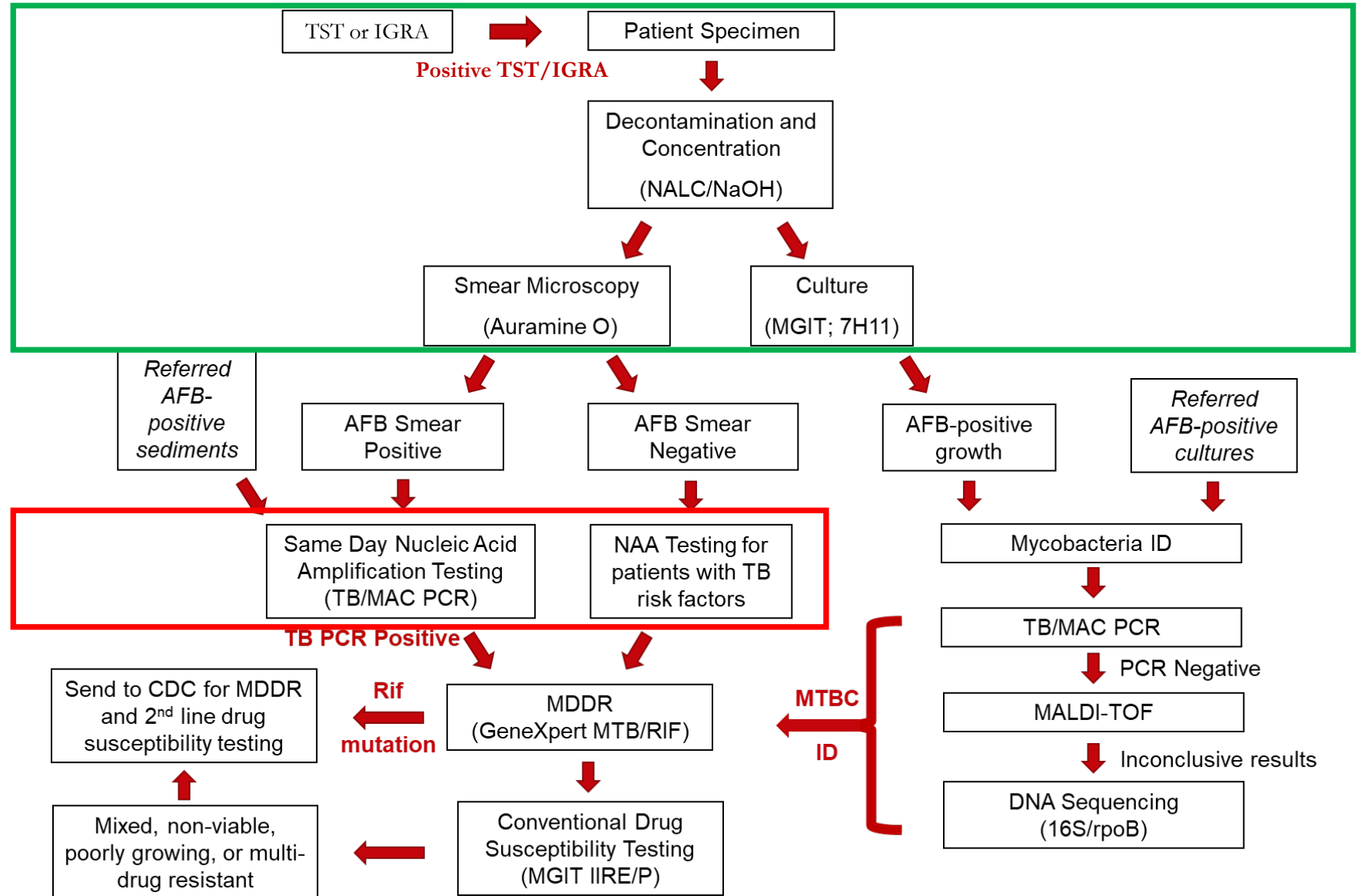
A close contact to a known TB-positive individual has a positive TST and clinical symptoms consistent with TB infection. Three sputum are collected and all three AFB smear are graded as AFB-positive, 1+/Rare. What testing, if any, would be best to determine whether the individual has TB present in their sputum?

- A. None needed, they have confirmed TB exposure and their sputum is positive for AFB
- B. Perform an IGRA since it's more specific for TB than the TST
- C. Perform AFB culture to see if TB grows
- D. GeneXpert or other nucleic acid amplification testing to rapidly determine presence or absence of TB

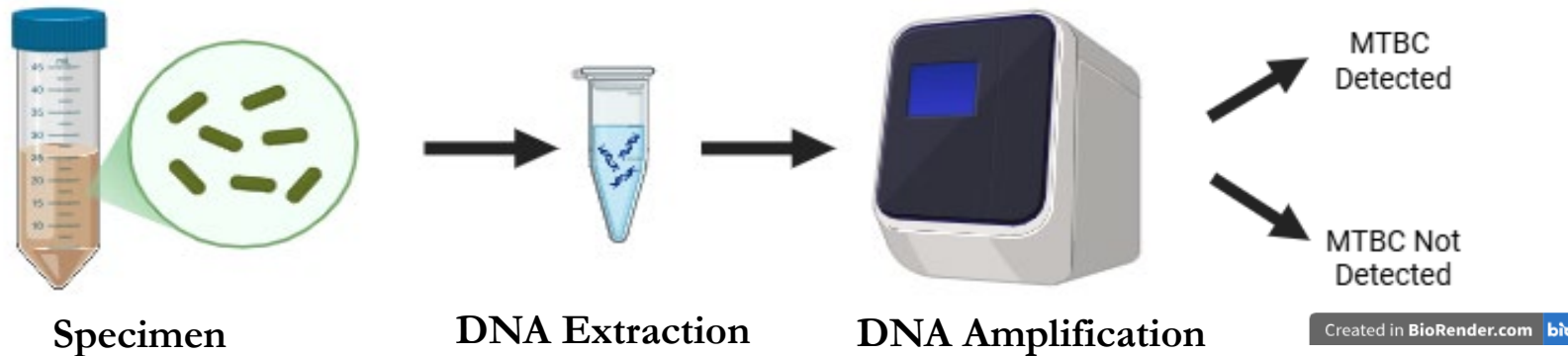
Modern Mycobacteriology

Rapid screen for *potential* MTBC infection

Rapid ID of MTBC from primary specimens



Rapid ID of MTBC from Primary Specimens – Nucleic Acid Amplification Testing (NAAT)



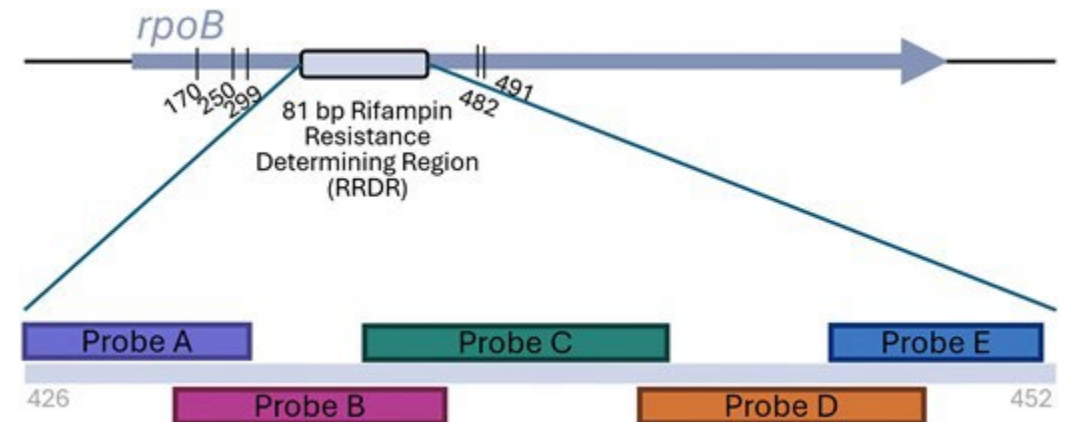
WSLH TB/MAC PCR (LDT) – Is MTBC DNA present in this specimen?

- Molecular target: IS6110; present in multiple copies in most MTBC
- Amplification = Sensitivity
 - Limit of detection: ~1 MTBC bacillus per PCR reaction
 - >99% for AFB smear-positive, culture-confirmed TB patients
- CDC recommendation: test first sputum of all patients suspected to have TB for whom the test result would alter case management or TB control activities
 - **Should not be routinely ordered when clinical suspicion of TB is low**

Rapid ID of MTBC from Primary Specimens – Nucleic Acid Amplification Testing (NAAT)

GeneXpert MTB/RIF

- Nested PCR assay
 - Target: *rpoB* gene RRDR (RIF-resistance determining region)
 - Presence of MTBC-specific *rpoB* sequence
 - Presence of *rpoB* mutations
- LOD: ≈ 130 AFB/ml
- FDA-approved for sputum only
 - All other specimen types require lab-specific validation



<https://academic.oup.com/ofid/article/12/4/ofaf132/8052695>

Rapid ID of MTBC from Primary Specimens – Nucleic Acid Amplification Testing (NAAT)



- Advantages
 - Speed: WSLH TB PCR, GeneXpert about 2 hr. to test; >80% resulted within 1 day of receipt
 - Sensitivity: WSLH TB PCR >99% for AFB smear-positive / TB-positive specimens
 - Specificity: Non-reactive to other mycobacteria, bacterial pathogens
 - Early prediction of drug resistance (GeneXpert, certain LDT PCR)
- Limitations
 - <50% of AFB smear-negative, culture-confirmed TB patients
 - Limits on acceptable specimen types for testing
 - Requires dedicated instrumentation and laboratory-specific validations
 - Expensive

A patient with a positive IGRA, suspicious chest X-ray, and hemoptysis has a BAL and a lung tissue biopsy collected for AFB smear/culture.

- AFB smears are negative on both

- GeneXpert on the BAL was negative

- Cultures have not gone positive at 17 days post-collection

Is it reasonable to assume this patient **does not** have active TB?

A. Yes, the negative smear and negative GeneXpert together exclude TB

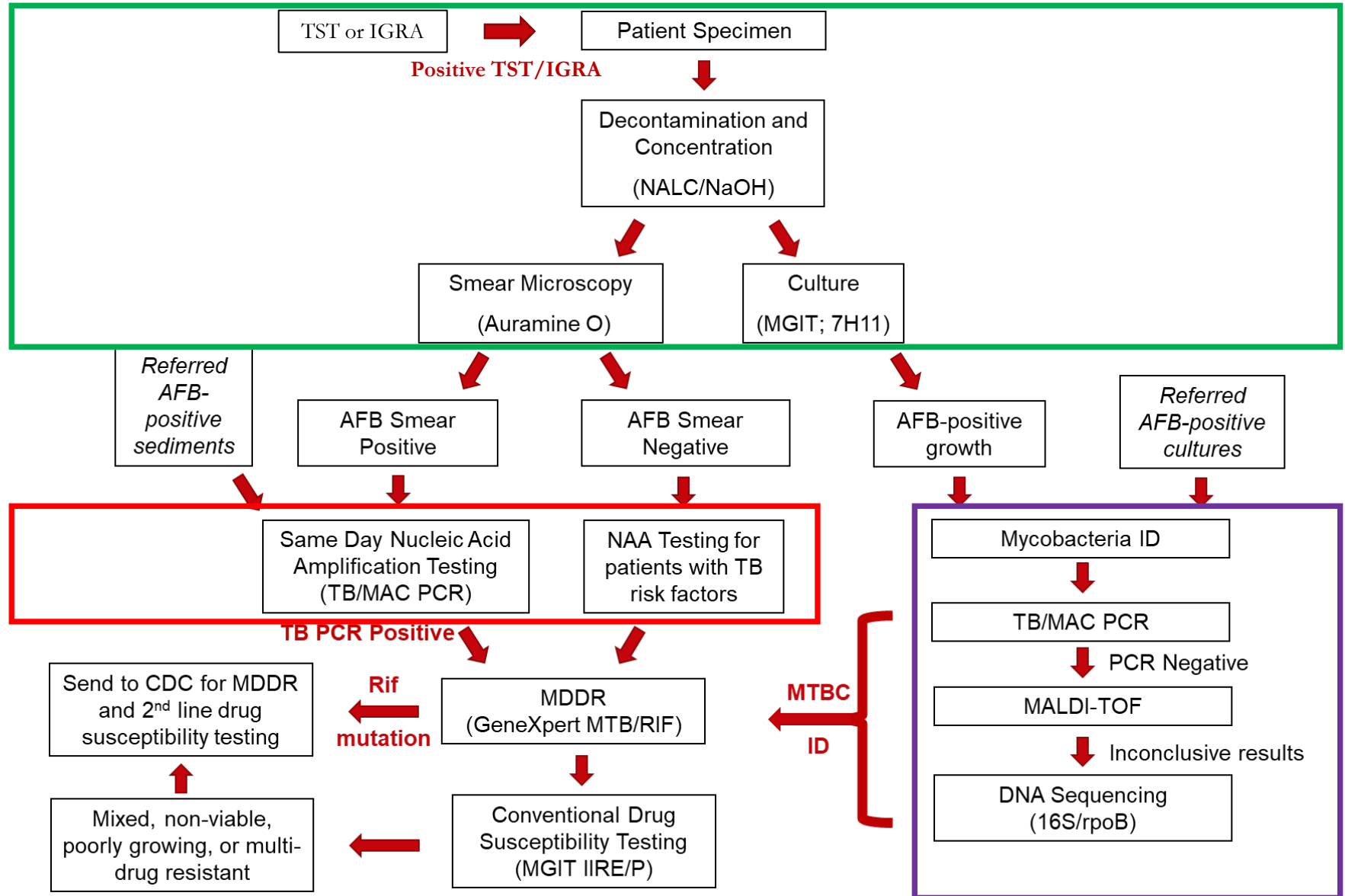
B. No, smear-negative specimens may be below assays' limits of detection. Culture results would be most accurate for this specimen type

C. Yes, a TB-positive culture should have gone positive well before 17 days

D. No, guidelines recommend at least 2 negative GeneXpert before ruling-out TB; run GeneXpert on the lung tissue before ruling out TB

Modern Mycobacteriology

Rapid screen for *potential* MTBC infection



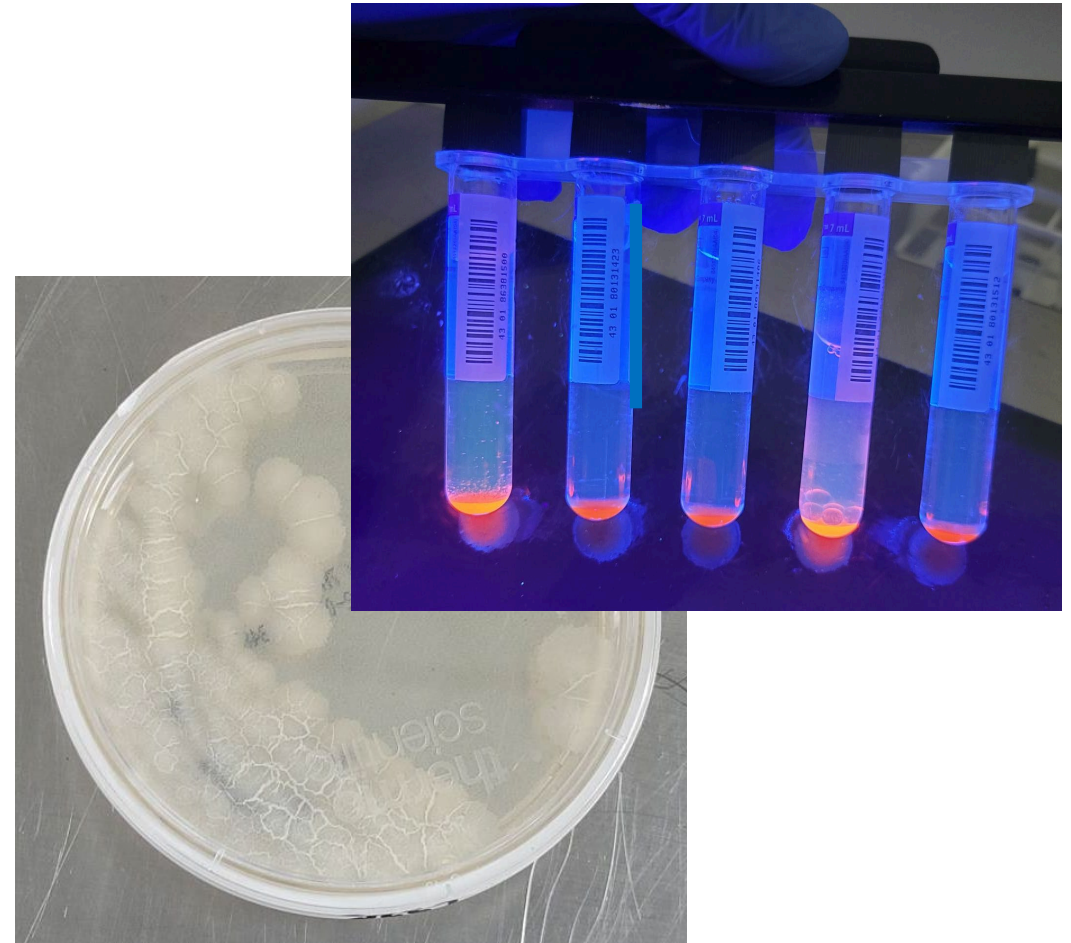
Rapid ID of MTBC from primary specimens

ID of MTBC from positive culture

ID of MTBC from positive culture

Mycobacterial culture – Is *viable* MTBC present in this patient specimen?

- “Gold standard”
 - Most sensitive method for mycobacterial detection (10 viable AFB/ml)
- Slowest method
 - Average time to detection: 15 days
 - Range: 4-40+ days
- Culture required for certain testing
 - Phenotypic DST
 - Genotyping (epidemiological linkages)



ID of MTBC from positive culture



Cepheid

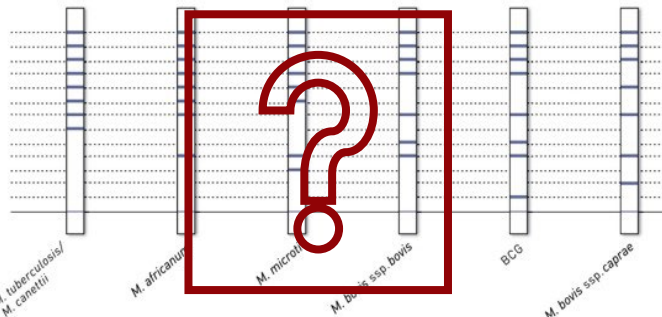


Bruker

HOLOGIC

**MYCOBACTERIUM TUBERCULOSIS
COMPLEX CULTURE IDENTIFICATION TEST**

Hologic



<https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/genotype-mtbc.html>



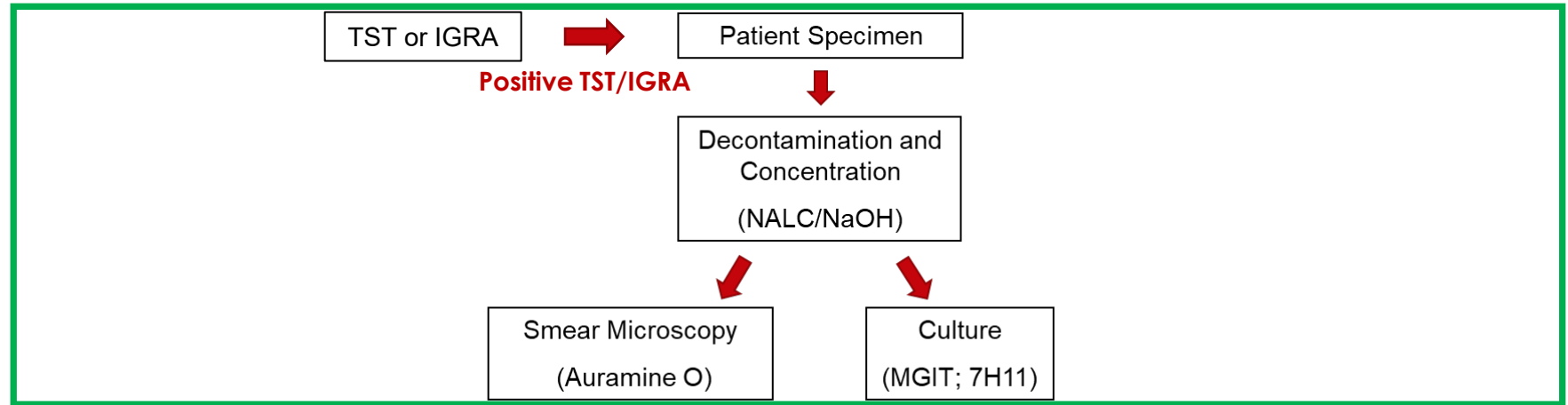
ThermoFisher

NAAT (LDT), MALDI-TOF, DNA sequencing (LDT), ?line probe assay (LDT)

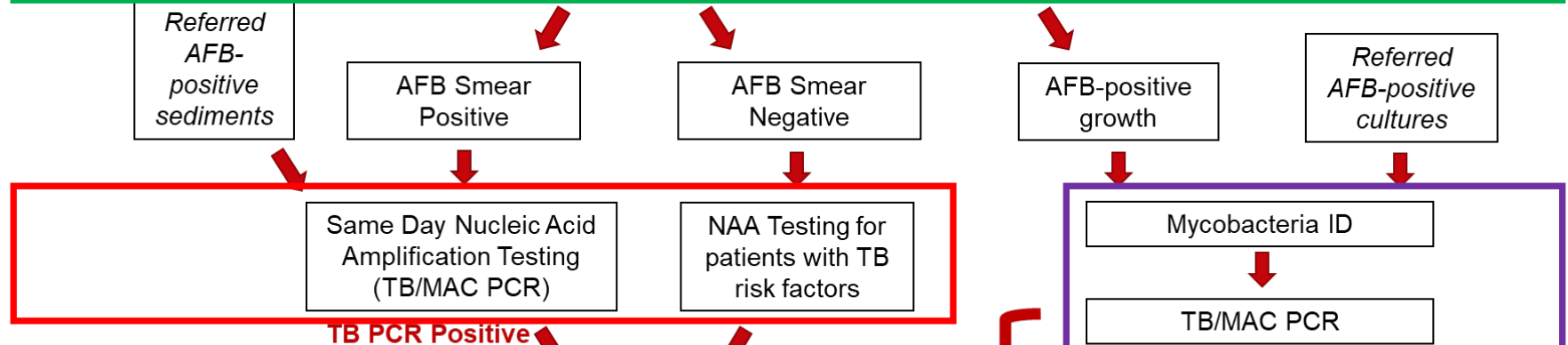
- Limitations:
 - May need pure subculture
 - List of FDA-approved MTB identification tests from culture:
 - 510K MALDI-TOF libraries
 - Access
 - Very limited availability of commercial products in US
 - Discontinuation of AFB testing products

Modern Mycobacteriology

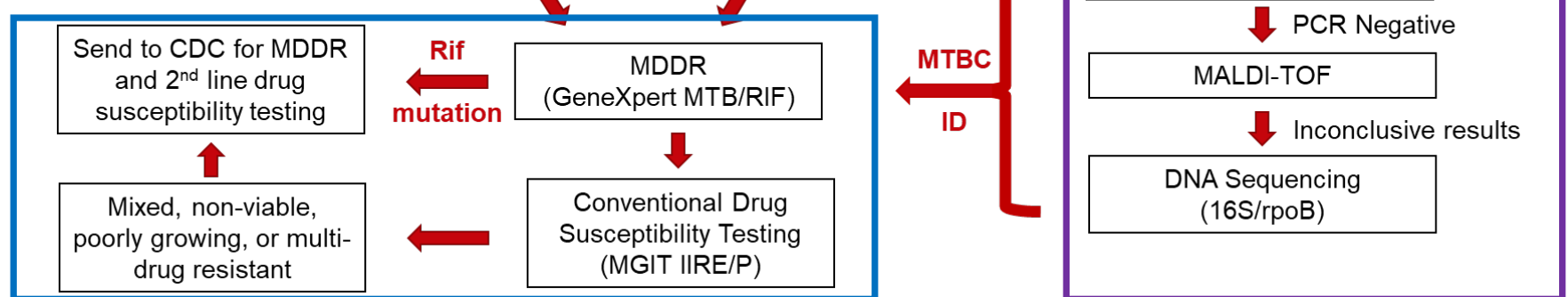
Rapid screen for *potential* MTBC infection



Rapid ID of MTBC from primary specimens



ID of MTBC from positive culture



Access to MTBC drug susceptibility testing

MTBC Drug Susceptibility Testing (DST)

RESEARCH

MTBC DST– What drug combination gives the highest chance for successful elimination of MTBC?

- Guide for choosing treatment plan — provide the best chance of a cure and lower development of resistance
- Initiate appropriate treatment for contacts of infectious cases

Worldwide Emergence of Extensively Drug-resistant Tuberculosis

https://wwwnc.cdc.gov/eid/article/13/3/06-1400_article

Fluoroquinolone Resistance in Drug-Resistant Tuberculosis, Kharkiv, Ukraine, 2019–2023

https://wwwnc.cdc.gov/eid/article/31/3/24-1675_article

Morbidity and Mortality Weekly Report

Outbreak of Multidrug-Resistant Tuberculosis — Kansas, 2021–2022

<https://www.cdc.gov/mmwr/volumes/72/wr/pdfs/mm7235a4-H.pdf>

DISPATCHES

Bedaquiline Resistance after Effective Treatment of Multidrug-Resistant Tuberculosis, Namibia

https://wwwnc.cdc.gov/eid/article/30/3/24-0134_article

A patient with clinical symptoms consistent with TB has sputum collected; sputum is AFB-positive and follow-up GeneXpert MTB/RIF testing indicates “MTB Detected, RIF resistance Detected”.

What follow-up testing, if any, do you think is necessary to determine whether this patient has rifampin-resistant TB?

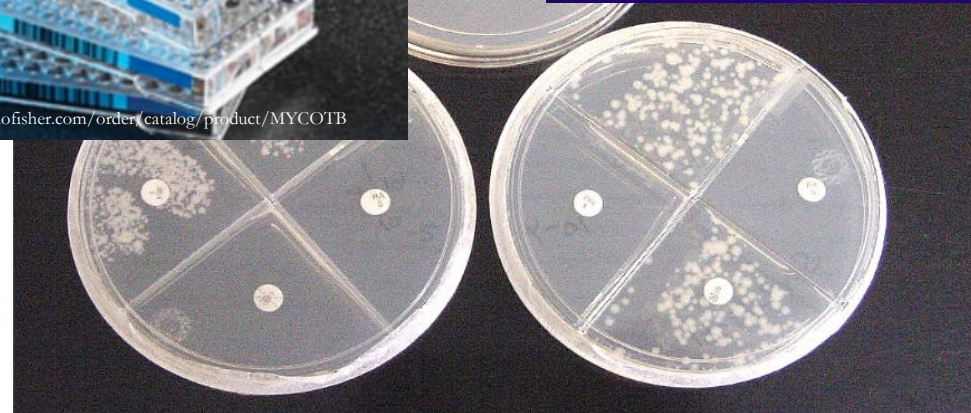
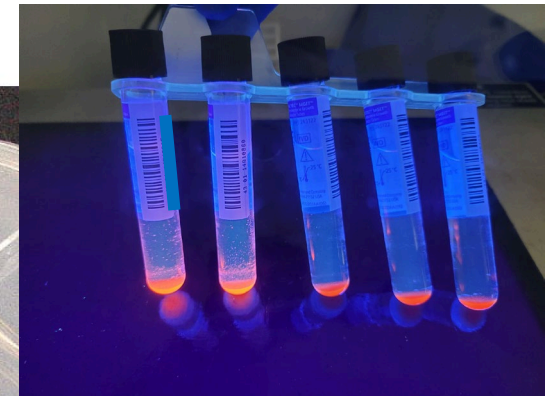
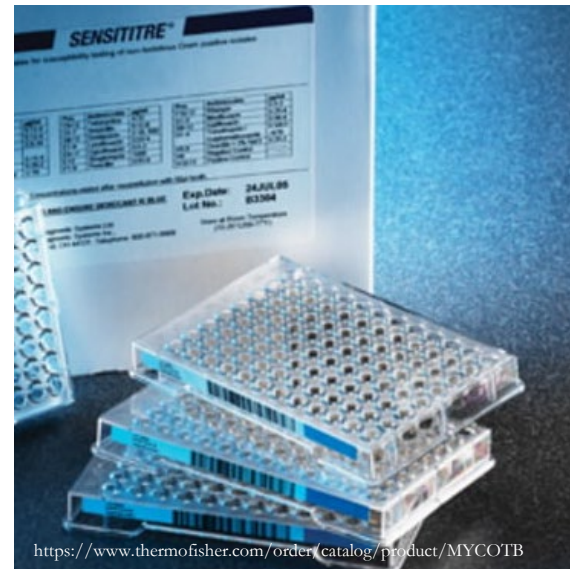
- A. None, the GeneXpert test looks for the most common rifampin resistance mutations in *rpoB* and has identified a resistance mutation
- B. Identify the specific *rpoB* mutation through DNA sequencing to determine if the mutation is linked to RIF resistance
- C. Perform culture-based rifampin susceptibility testing to determine whether the isolate is functionally resistant to RIF
- D. Some combination of B and C, depending on specimen material available

MTBC Drug Susceptibility Testing: Phenotypic

BD MGIT, Versatrek, Sensititre MTB, agar proportion

Phenotypic DST: Does the isolate grow in the presence of drug?

- Advantages:
 - “Gold standard” test
 - Limited FDA-approval
 - Test variety of drugs, concentrations
- Limitations:
 - Requires **viable pure** culture
 - Critical concentration updates?
 - Reagent shortages

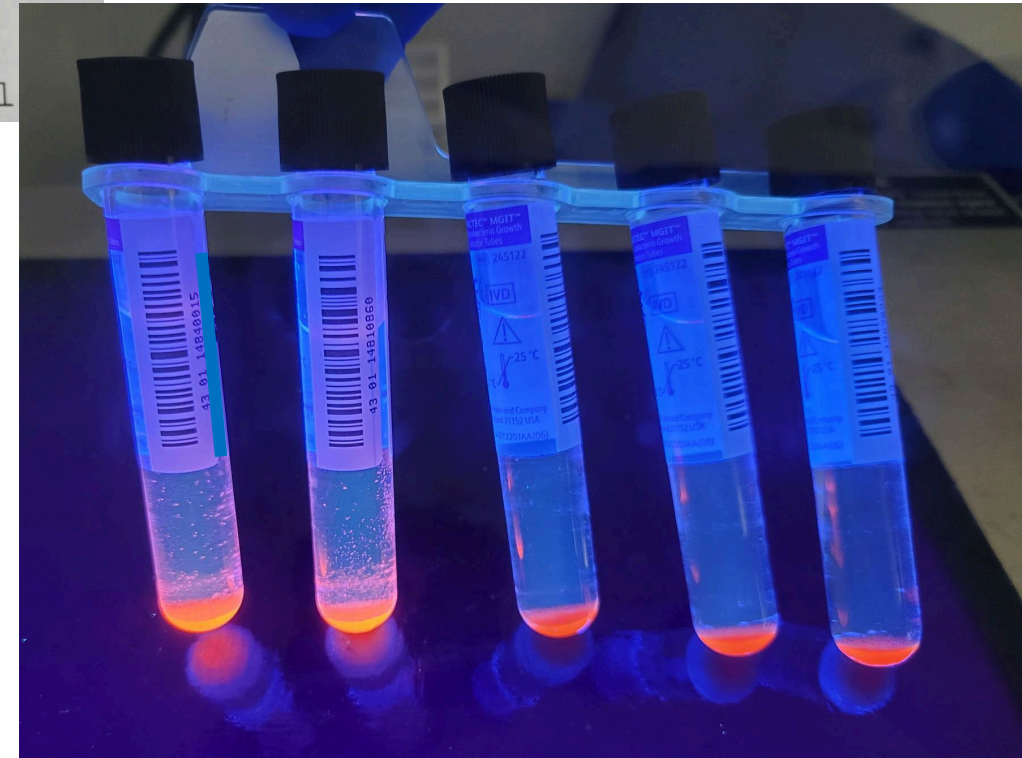


<https://www.aphl.org/conferences/proceedings/Documents/2008/TB-Conference/005-Metchock.pdf>

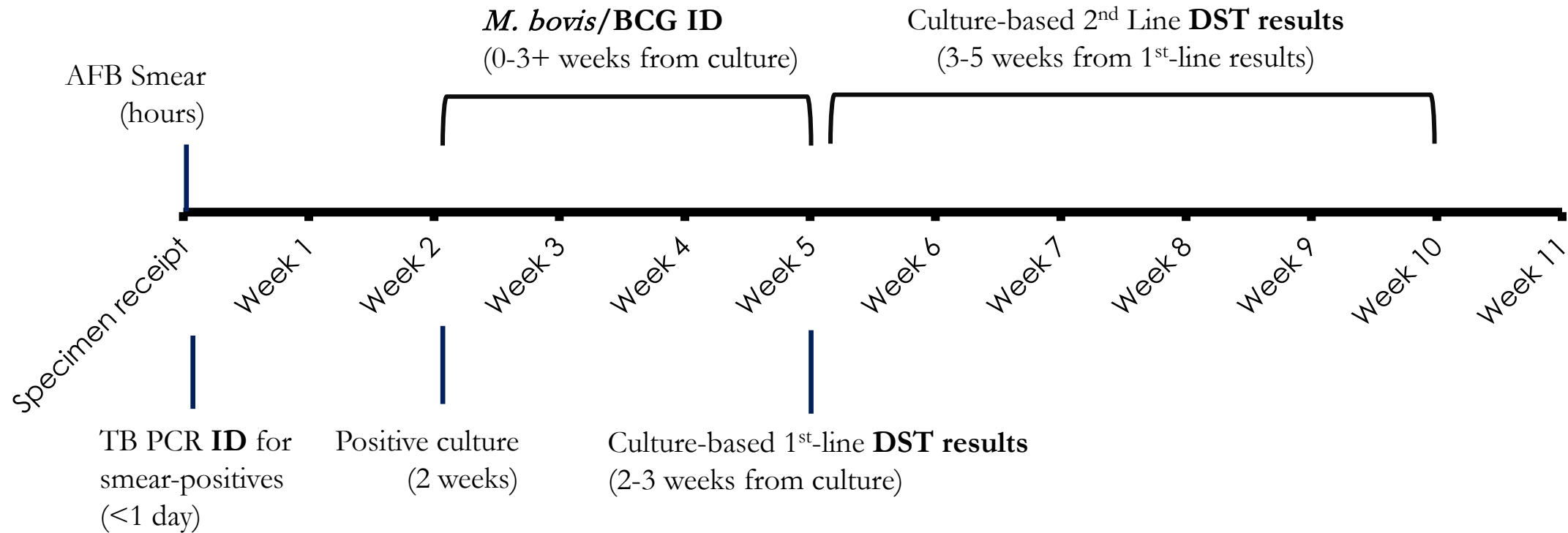
MTBC Drug Susceptibility Testing: Phenotypic

Sequence No: 4395 [REDACTED]		TIP:10;15		SOP: [REDACTED]	Removed
Access No: 26MM0 [REDACTED]		Isolate No: 1			
Tube Position	Growth Unit	Status	Concentration	Drug Name	
A/E03	400	C		Growth Control	
A/E04	400	R	0.10 ug/mL	Isoniazid	
A/E05	0	S	0.40 ug/mL	Isoniazid	
A/E06	0	S	1.00 ug/mL	Rifampin	
A/E07	0	S	5.00 ug/mL	Ethambutol	

- Incubate a standardized concentration of MTBC isolate with a defined concentration of a drug
 - Growth control provides evidence organism is viable
 - If resistant, isolate will grow in presence of critical concentration of drug



Phenotypic testing for TB drug resistance is sloooooow

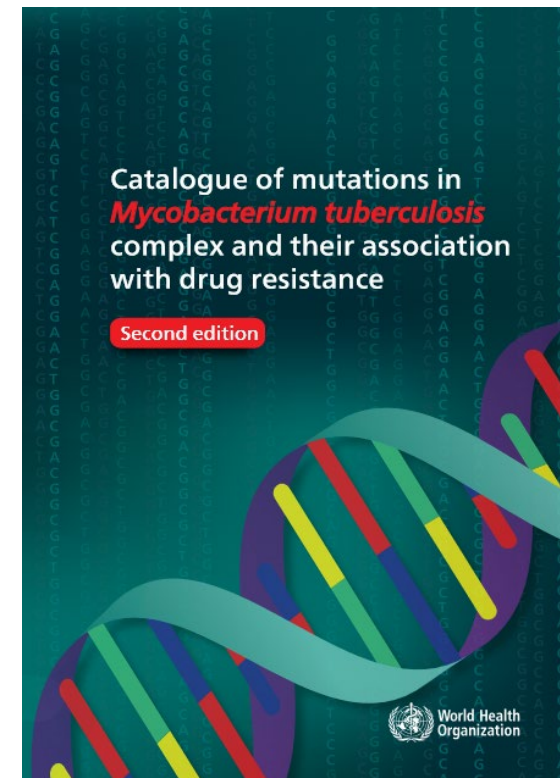


MTBC Drug Susceptibility Testing: Molecular

Next-generation sequencing (NGS),
Sanger DNA sequencing, GeneXpert

Molecular DST: Are there mutations present known or suspected to cause drug resistance?

- Advantages:
 - Can analyze mixed, non-viable cultures
 - Possible to screen entire MTBC genome
 - Much quicker than culture
- Limitations:
 - Requires DNA isolation
 - Requires knowledge of drug-resistance mutations
 - What to do with unknown or unlinked mutations?
 - Higher level sequencing requires bioinformatics know-how

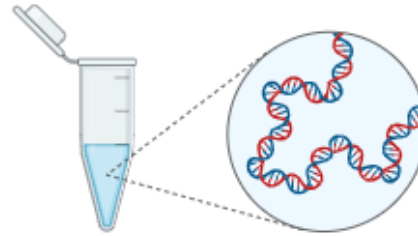


MTBC Drug Susceptibility Testing: Molecular

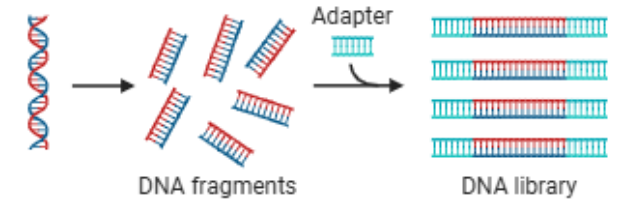
Next-generation sequencing (NGS)

- Millions of short DNA strands ('reads') per specimen
- Whole Genome Sequencing (WGS) vs targeted NGS (tNGS)
 - Time
 - Cost
 - Specimen type
 - Assay design and complexity
 - Computing power/analysis

Step 1:
DNA extraction

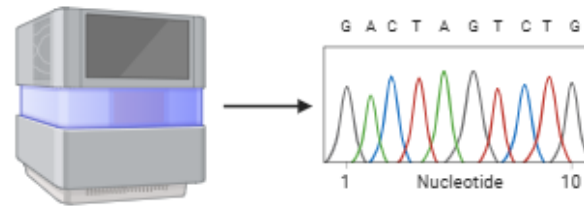


Step 2:
Library preparation



Next Generation Sequencing Workflow

Step 3:
Sequencing



Step 4:
Analysis



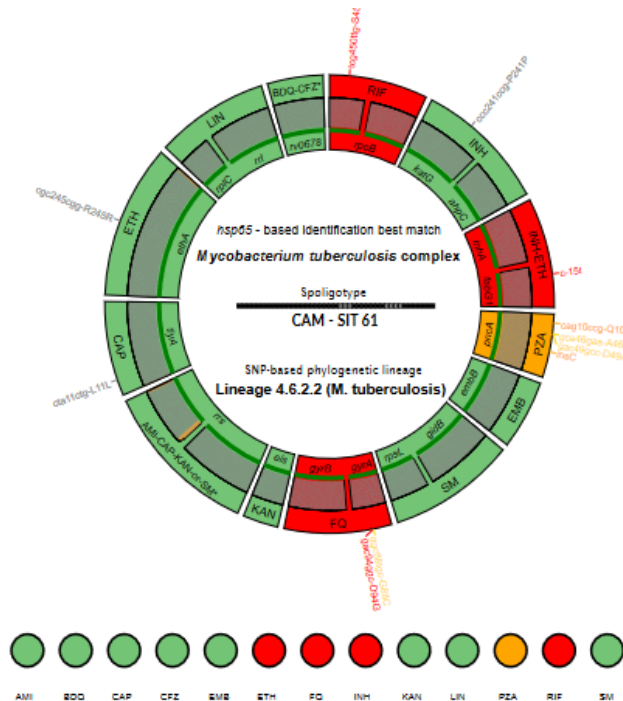
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MTBC Drug Susceptibility Testing: tNGS



MTBC tNGS kits

- Pre-made primer sets to amplify TB gene of interest from sputum or culture
- Sequenced on NGS sequencing platforms
- Analysis through proprietary software to identify organism (species), mutations in resistance genes



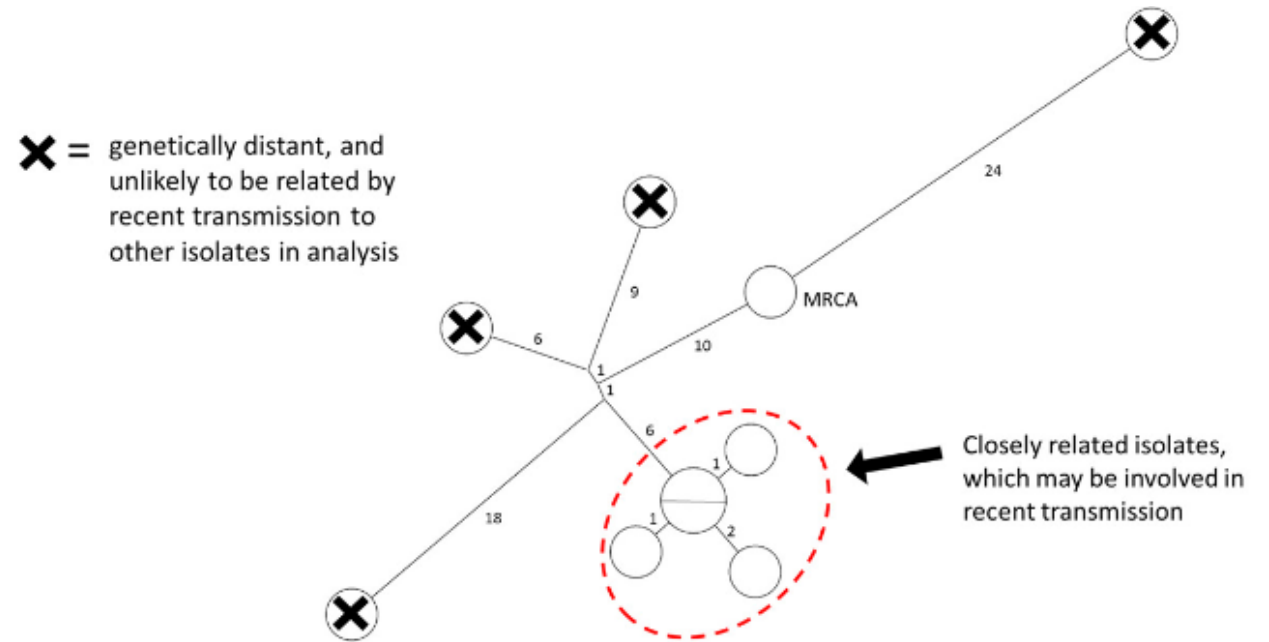
- GenoScreen Deeplex Myc-TB
 - Amplifies 18 MTBC resistance genes, ID, spoligotype
 - INH, RIF, EMB, PZA, FQ, LZD, BDQ, CFZ, AMK, STR, ETH, KAN, CAP

<https://www.deeplex.com/deeplex-myc-tb/>

WISCONSIN STATE LABORATORY OF HYGIENE - UNIVERSITY OF WISCONSIN

MTBC Epidemiology: MTBC Genotyping with wgMLST

- In 2018, National TB Molecular Surveillance Center began universal whole-genome sequencing
- wgMLSType analysis covers 70% of genome
 - Isolates that have <7 SNP are assigned a wgMLSType
 - Isolates with >7 SNP are assigned “MTBCUnique”
- Allows for creation of phylogenetic trees to visualize relatedness of isolates; enhances granularity to more reliably identify closely-related isolates



<https://www.cdc.gov/tb/programs/genotyping/pdf/wgMLST-Training-Webinar>

MTBC Epidemiology: WI MTBC Clusters

State Case Number	County	GENType	PCRType	State Cluster Name	wgMLSType
2000WII000000023			PCR00041	WI_0010	
2002WII000020034			PCR00041	WI_0010	
2002WII000020055			PCR00041	WI_0010	
2002WII000020069			PCR00041	WI_0010	
2002WII000020072			PCR00041	WI_0010	
2002WII000020077			PCR00041	WI_0010	
2003WII000030007			PCR00041	WI_0010	
2004WII000040038		G05054	PCR00041	WI_0010	
2005WII000050016		G04988	PCR00041	WI_0010	
2005WII000050011		G04878	PCR00041	WI_0010	
2006WII000060018		G05134	PCR00041	WI_0010	
2006WII000060035		G04850	PCR00041	WI_0010	
2008WII000080032		G04888	PCR00041	WI_0010	
2010WII000100049	RACINE	G05020	PCR00041	WI_0010	
2011WII000110070	BROWN	G04944	PCR00041	WI_0010	
2005WII000120045		G00016	PCR00041	WI_0010	
2007WII000130013		G00016	PCR00041	WI_0010	
2012WII000120070		G00016	PCR00041	WI_0010	
2015WII000150038	WAUKESHA	G05083	PCR00041	WI_0010	
2015WII000150065	SHEBOYGAN	G04942	PCR00041	WI_0010	
2016WII000160014	MARATHON	G04811	PCR00041	WI_0010	
2016WII000160019	MILWAUKEE	G04958	PCR00041	WI_0010	
2018WII000180020	KENOSHA	G39973	PCR00041	WI_0010	MTBCunique
2019WII000190025	WAUKESHA	G04920	PCR00041	WI_0010	MTBCunique
2019WII000190042	MILWAUKEE	G05055	PCR00041	WI_0010	MTBCunique
2020WII000200008	MILWAUKEE	G05006	PCR00041	WI_0010	MTBCunique
2021WII000210033	MILWAUKEE	G05014	PCR00041	WI_0010	MTBCunique
		G00016	PCR00041	WI_0010	MTBC002635
2022WII000220006	OUTAGAMIE	G05044	PCR00041	WI_0010	MTBCunique
2022WII000220023	KENOSHA	G05056	PCR00041	WI_0010	MTBCunique

State Case Number	County	GENType	PCRType	State Cluster Name	wgMLSType
2010WII0002010037	DANE	G01449	PCR00002	WI_0056	
2011WII000211040	MILWAUKEE	G01449	PCR00002	WI_0056	
2011WII000211046	MILWAUKEE	G01449	PCR00002	WI_0056	
2011WII000211050	MILWAUKEE	G01449	PCR00002	WI_0056	
2011WII000211016	DANE	G01086	PCR00002	WI_0056	
2012WII000212009	BROWN	G17490	PCR00002	WI_0056	
2012WII000212029	MILWAUKEE	G01204	PCR00002	WI_0056	
2012WII000212040	DANE	G01322	PCR00002	WI_0056	
2012WII000212062	MILWAUKEE	G01198	PCR00002	WI_0056	
2012WII000212060	SHEBOYGAN	G01449	PCR00002	WI_0056	
2012WII000212025	MILWAUKEE	G01449	PCR00002	WI_0056	
2013WII000213007	MILWAUKEE	G00010	PCR00002	WI_0056	
2013WII000213017	DANE	G01363	PCR00002	WI_0056	
2013WII000213040	MARATHON	G01449	PCR00002	WI_0056	
2014WII000214018	MILWAUKEE	G01190	PCR00002	WI_0056	
2014WII000214044	LA CROSSE	G01449	PCR00002	WI_0056	
2015WII000215015	MILWAUKEE	G01437	PCR00002	WI_0056	
2015WII000215025	DANE	G29837	PCR00002	WI_0056	
2015WII000215026	EAU CLAIRE	G01449	PCR00002	WI_0056	
2015WII000215053	WINNEBAGO	G01449	PCR00002	WI_0056	
2015WII000215066	OUTAGAMIE	G01449	PCR00002	WI_0056	
2016WII000216010		G00012	PCR00002	WI_0056	
2016WII000216038	JUNEAU	G01284	PCR00002	WI_0056	
2017WII000217019	LA CROSSE	G01448	PCR00002	WI_0056	
2018WII000218021	FOND DU LAC	G39972	PCR00002	WI_0056	
2018WII000218017	MILWAUKEE	G00010	PCR00002	WI_0056	MTBC004348
2018WII000218021	FOND DU LAC	G39972	PCR00002	WI_0056	MTBCunique
2018WII000218034	SHEBOYGAN	G01449	PCR00002	WI_0056	MTBC000218
2019WII000219045	DANE	G01121	PCR00002	WI_0056	MTBC004348
2020WII000220005	SHEBOYGAN	G01449	PCR00002	WI_0056	MTBCunique
2019WII000219010	MILWAUKEE	G00010	PCR00002	WI_0056	MTBC017110
2019WII000219012	DANE	G00012	PCR00002	WI_0056	MTBC000218
2020WII000220011	DANE	G00010	PCR00002	WI_0056	MTBCunique
2021WII000221028	MARATHON	G01253	PCR00002	WI_0056	MTBC000218
2021WII000221045	MARATHON	G01253	PCR00002	WI_0056	MTBC002243
2021WII000221041	MARATHON	G01449	PCR00002	WI_0056	MTBCunique
2021WII000221001	MILWAUKEE	G01265	PCR00002	WI_0056	MTBC017110
2022WII000222014	MILWAUKEE	G01437	PCR00002	WI_0056	MTBC000218

MTBC Diagnostics: So, What Do You Know Now?

- Large portion of MTBC diagnostic testing is unchanged since the 1900s
 - MTBC culture is still the king!
- Accessing new MTBC testing is becoming increasingly difficult for US laboratories
 - Many new platforms or tests not available in US
 - About as many tests have been discontinued in US over the past 10 years have come to market
- Molecular testing is becoming increasingly useful
 - Faster, cheaper
 - Much more data available
 - Be careful with interpretation
- Labs are your partners!
 - Build relationships, communicate, and work with them to improve testing and patient care!



**Wisconsin State
Laboratory of Hygiene**
UNIVERSITY OF WISCONSIN-MADISON

WSLH Communicable Disease Division

Allen Bateman
Alana Sterkel
Laura Louison
Michael Mamerow
CDD LIMS Admins

WSLH Mycobacteriology Laboratory

Carter Seehafer
Chrystal Egstad
Kristen Wendtland
Lindsey Moderski
Nicholas Mack

WSLH Sequencing

Alicia Mooney
Ava Gehrke-Laundrie
Claire LaFleur

WSLH Bioinformatics

Kelsey Florek
Abigail Shockey
Madeline Topf (APHL)



**WISCONSIN DEPARTMENT
of HEALTH SERVICES**

WI DHS TB Program

Claire Leback
Andrea Liptack
Mary Raschka



Mayo Clinic Center for Tuberculosis

**WI Mycobacteriology Laboratory Network
WI Clinical Laboratory Network**



Centers for Disease Control and Prevention

Department of TB Elimination
DTBE TB Laboratory



Association of Public Health Laboratories