Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

Not Taking the Long Road: Accelerating Diagnostic Testing for *Mycobacterium tuberculosis* Complex

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06/23/26

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

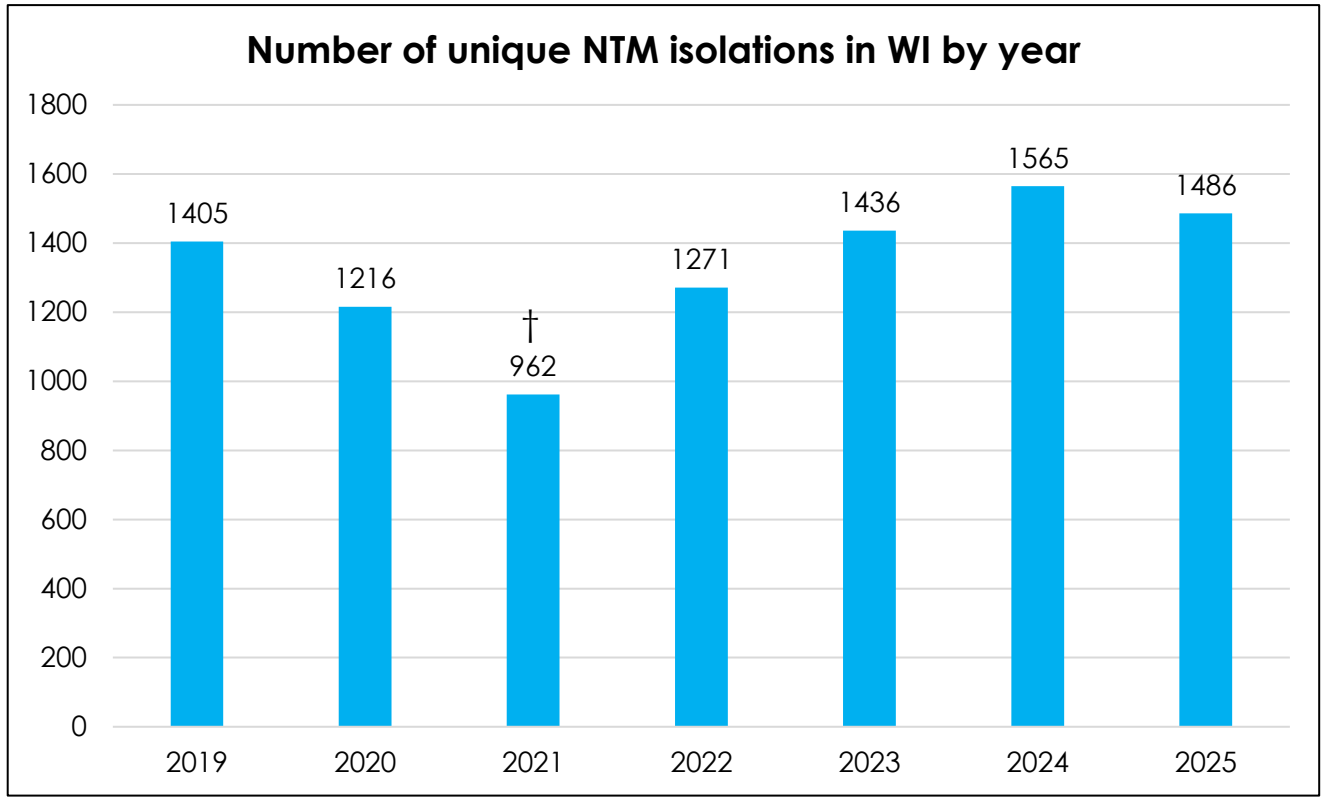
- *Mycobacterium tuberculosis* complex (MTBC)
- MTBC diagnostic testing
  - Historical testing
  - "Modern" testing
    - Rapid screening
    - MTBC ID from primary specimens
    - Culture ID
    - Susceptibility testing
  - Benefits of MTBC molecular diagnostics

Wisconsin Mycobacteriology Laboratory Network (WMLN)

- Started in 1998 as offshoot of WCLN
  - Mission: *Ensure state-wide access to rapid and reliable TB testing*
- Coordinated by WSLH; collaboration between public health and clinical labs, local and state public health, and other healthcare professionals
  - Infrastructure for communication
  - Education and outreach activities
  - Assessment of state lab capacity
  - Annual conference
  - **Laboratory-based surveillance**

WMLN: Non-Tuberculous Mycobacteria Epidemiology

Wisconsin Mycobacteriology Laboratory Network Data Report | **2025**



NTM isolations are self-reported to the Wisconsin State Laboratory of Hygiene by mycobacteriology labs in the state of Wisconsin. These data represent one unique isolation per organism per patient.

†Please note: Isolate data for 2021 is incomplete, as one WMLN lab was unable to submit NTM isolation data that year.

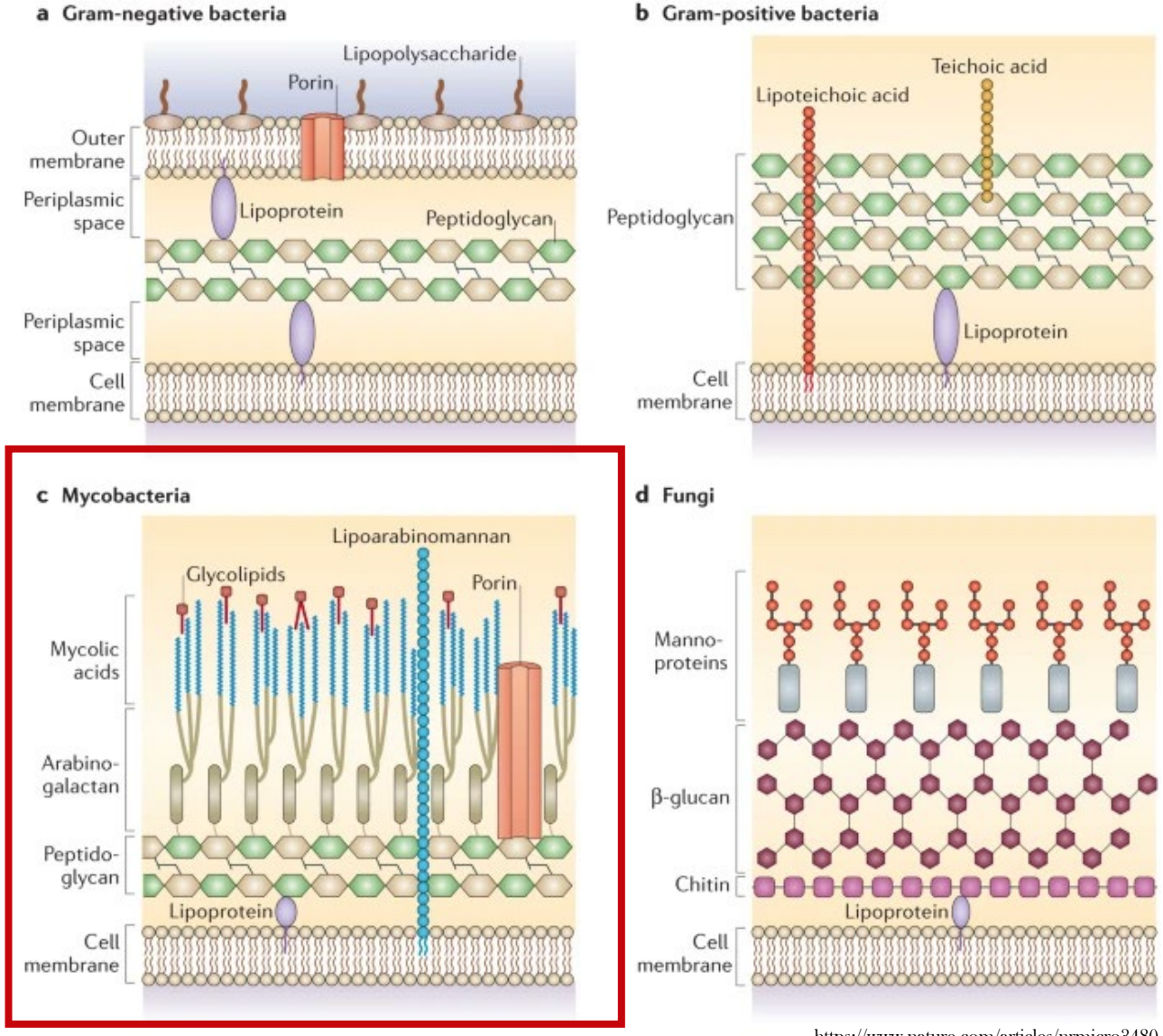
NTM Infection rate

APHL/CDC four state pilot study (2020): 3.8 per 100,000 population

WI (2025): 25 per 100,000

Mycobacteria, aka Acid-Fast Bacilli (AFB)

- (Very) slowly growing
  - Diagnosis
  - Treatment
- Specialized cell wall
  - Survival
  - Treatment
  - Staining
- Environmental pathogens*
  - <200 species
  - Infection generally via aerosol
  - **Except MTBC*



https://www.nature.com/articles/nrmicro3480
Nature Reviews | Microbiology

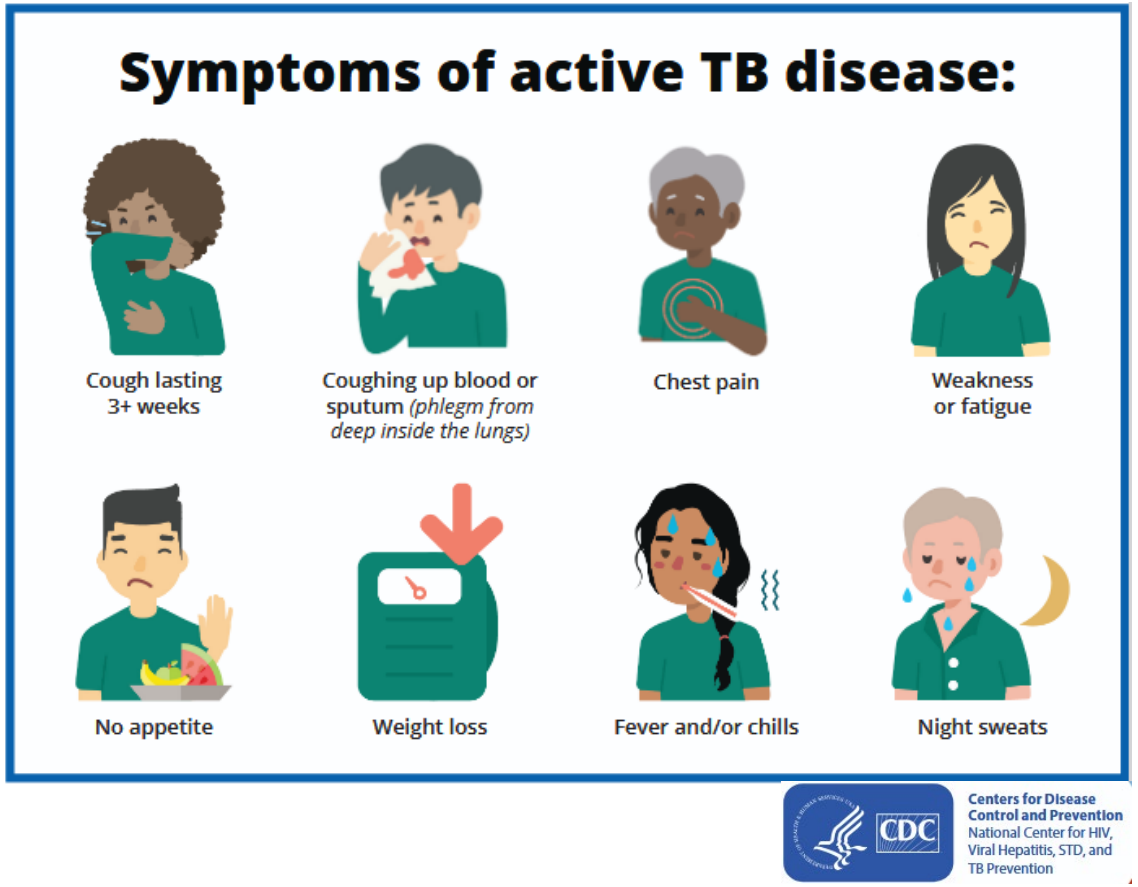
Tuberculosis: Intersection of Clinical and Public Health Practice

- Disease caused by infection with MTBC organisms
  - Includes *M. tuberculosis, M. bovis, M. bovis*-BCG (*M. caprae, M. microti, M. africanum, M. canettii*, *M. pinnipedii, M. mungi)*
  - *M. bovis* and *M. bovis*-BCG should be differentiated if suspected
- MTBC can enter and survive within macrophages, enter circulatory/lymph systems
- MTBC can be isolated from any body site
  - 2016-2024: lymph node, pleural, neck, urine, CSF, peritoneal, back, blood, chest, spine, hip, shoulder, stool, skin, pelvic fluid, pancreas, bone marrow, colon, gastric aspirate

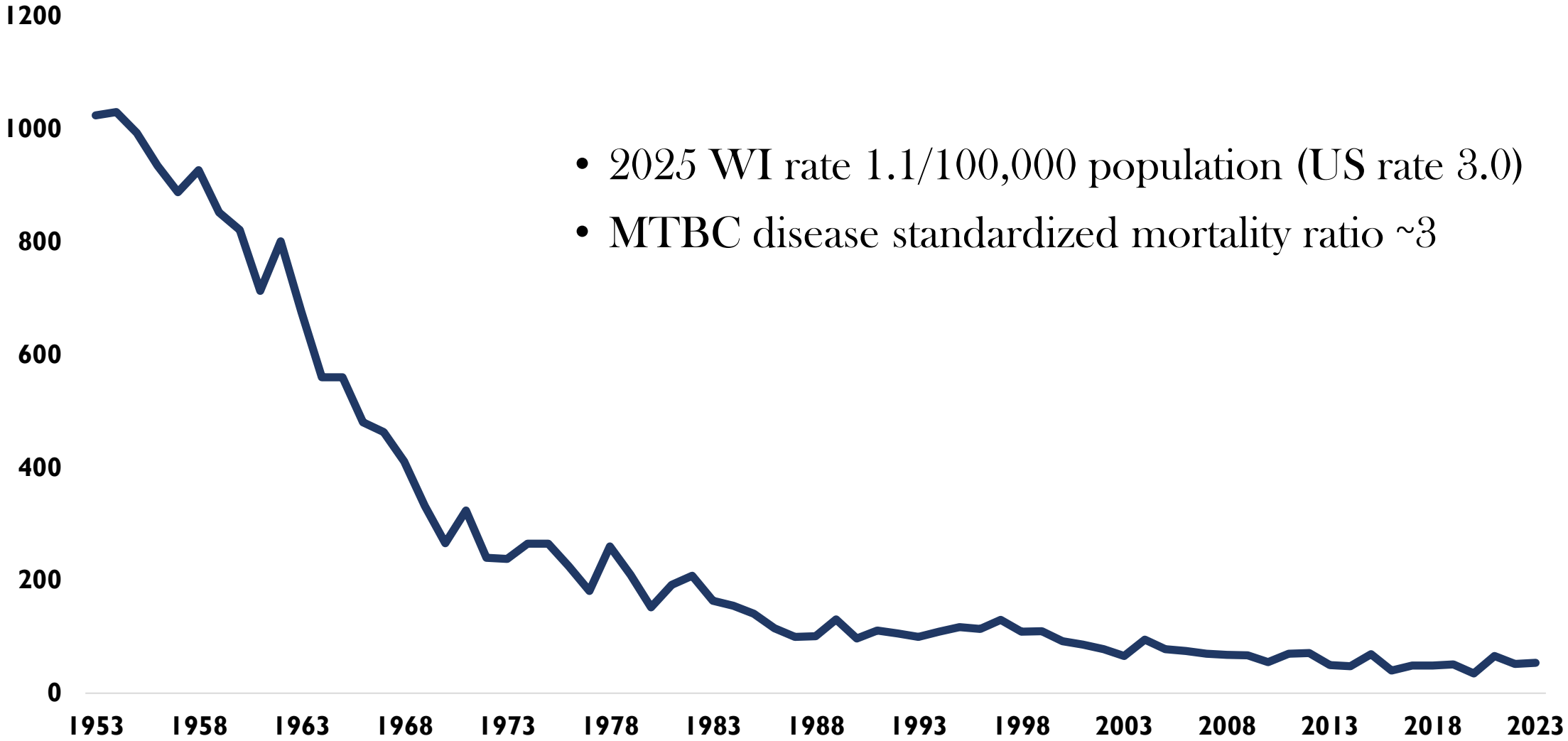
https://www.nature.com/articles/d41586-022-02987-z

Tuberculosis: Intersection of Clinical and Public Health Practice

- Leading cause of death from single infectious disease world-wide; 10.7m cases, 1.2m deaths (2024)
- Transmittable person-to-person via aerosol/droplet
  - ID_{50}: <10 bacilli
- Reportable disease in WI as of 1905
  - Active (Class I) and Latent (Class II)
- MTBC can rapidly develop drug resistance with inappropriate treatment
  - Standard treatment: 4 drug combination



Reported cases of TB have decreased dramatically since 1953



- 2025 WI rate 1.1/100,000 population (US rate 3.0)
- MTBC disease standardized mortality ratio ~3

Infection Cleared ?%

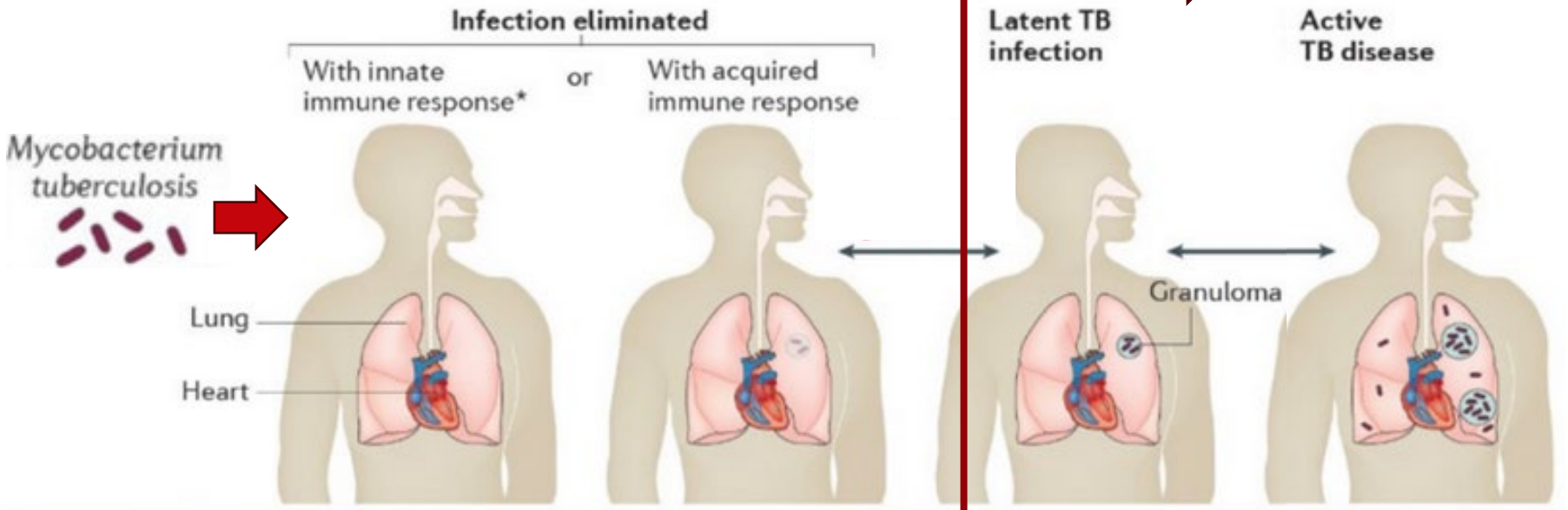
Infected

90%

LTBI

10%

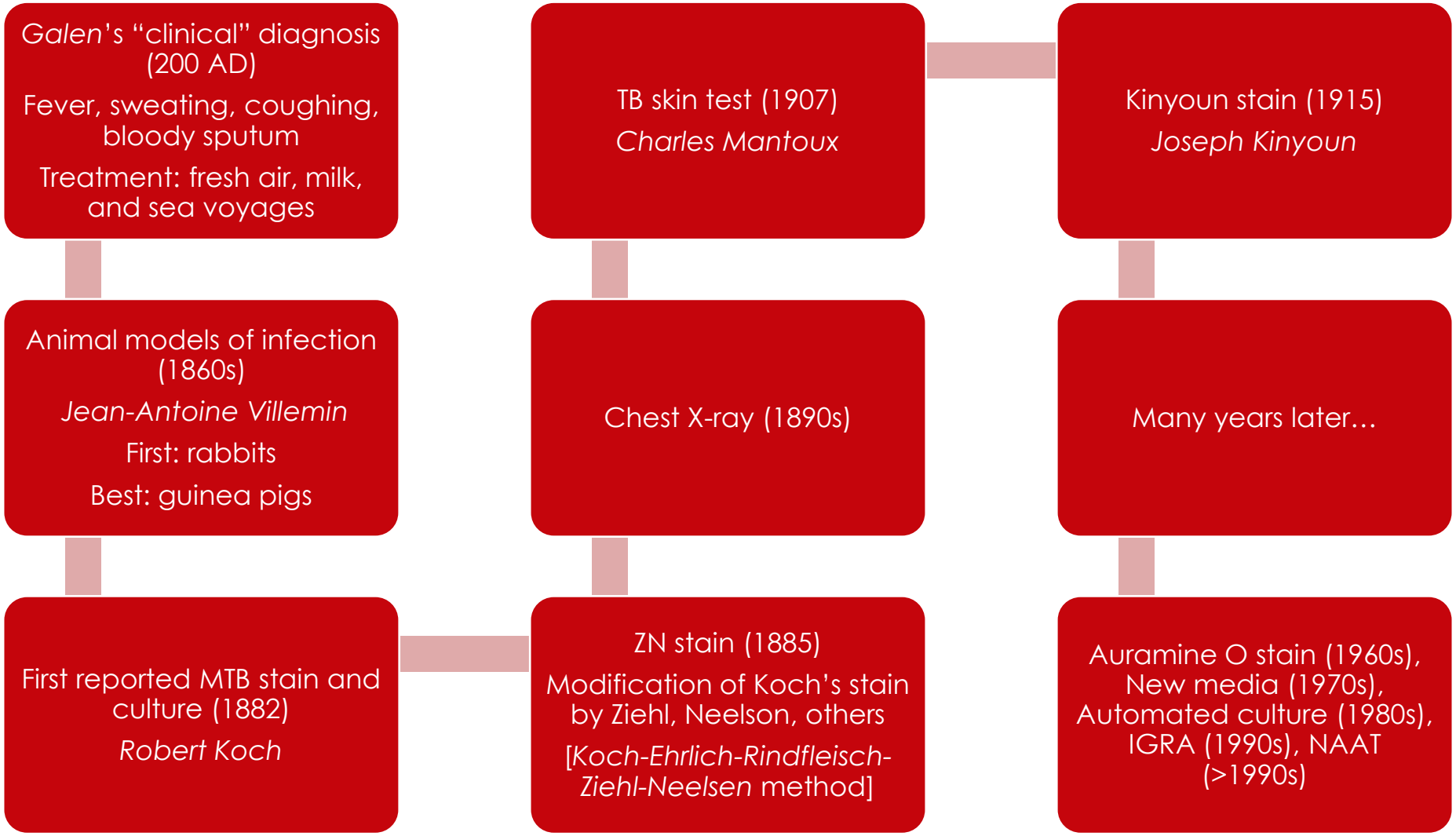
5-10%



TST	Negative	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Positive
Sputum smear	Negative	Negative	Negative	Positive or negative
Infectious	No	No	No	Yes
Symptoms	None	None	None	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy

Modified from: https://www.nature.com/articles/nrdp201676

MTBC diagnostics: Older than dirt



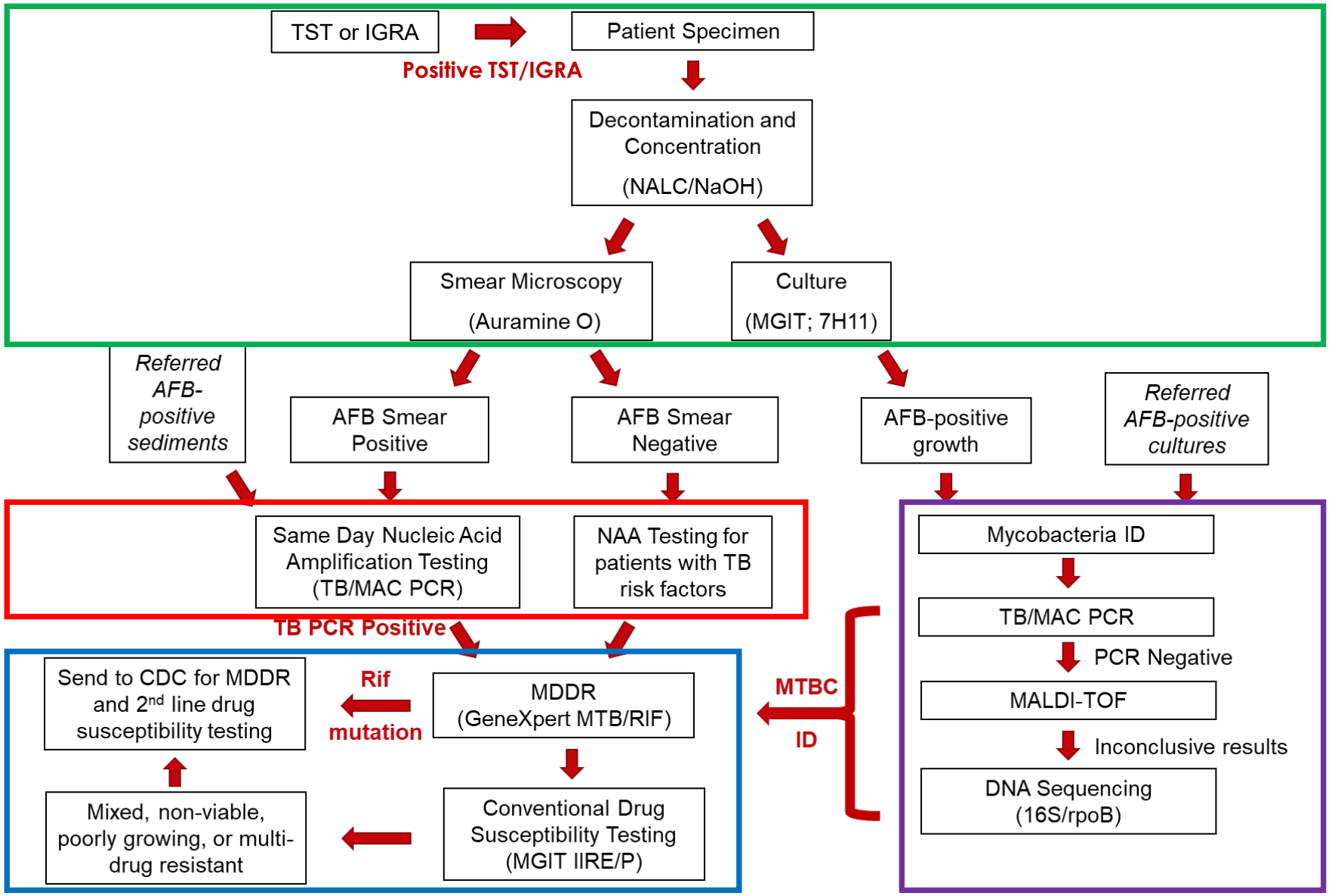
Modern Mycobacteriology

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



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 (mostly) for MTBC

- Limitations:
  - Requires multiple visits (TST) or laboratory (IGRA)
  - Some cross-reactivity with TST
  - Not ideal for those with BCG vaccine
  - Require competent immune system

Revvity

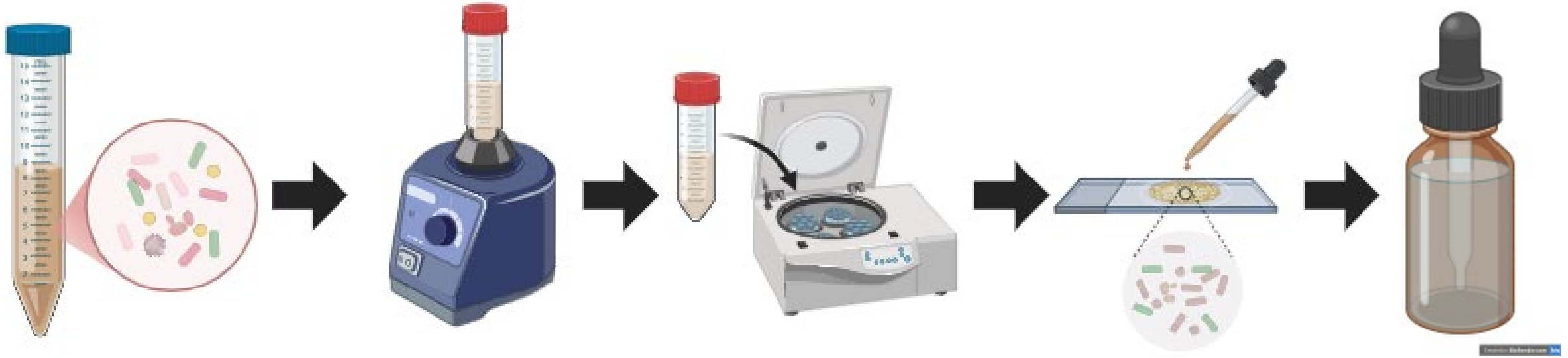
Sanofi

QIAGEN

Rapid screen for *potential* MTBC infection

AFB smear testing – Are there AFB present in primary clinical specimen?

Respiratory specimen → Decontaminate: NALC/NaOH → Centrifugation concentration → Prepare AFB smear from 1-2 drops concentrated sediment → Stain slides



- 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

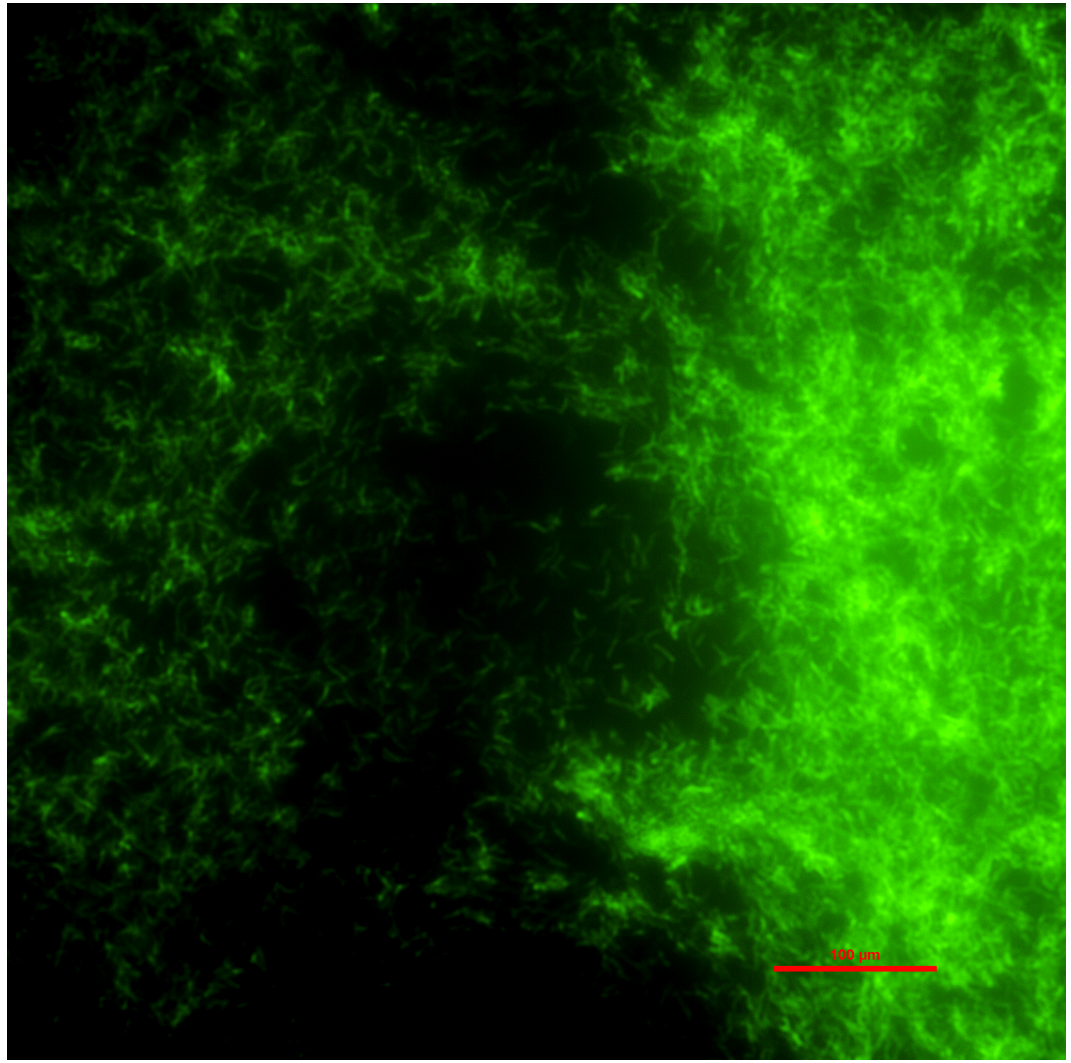
- One slide per patient to prevent cross-contamination

- 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:
  - Limited specificity
  - Limited sensitivity (<50%)
  - Does not indicate viability



And then, an eternity passes (relatively speaking)

Positive culture
(2+ weeks)

Positive AFB Smear
(<24 hours)

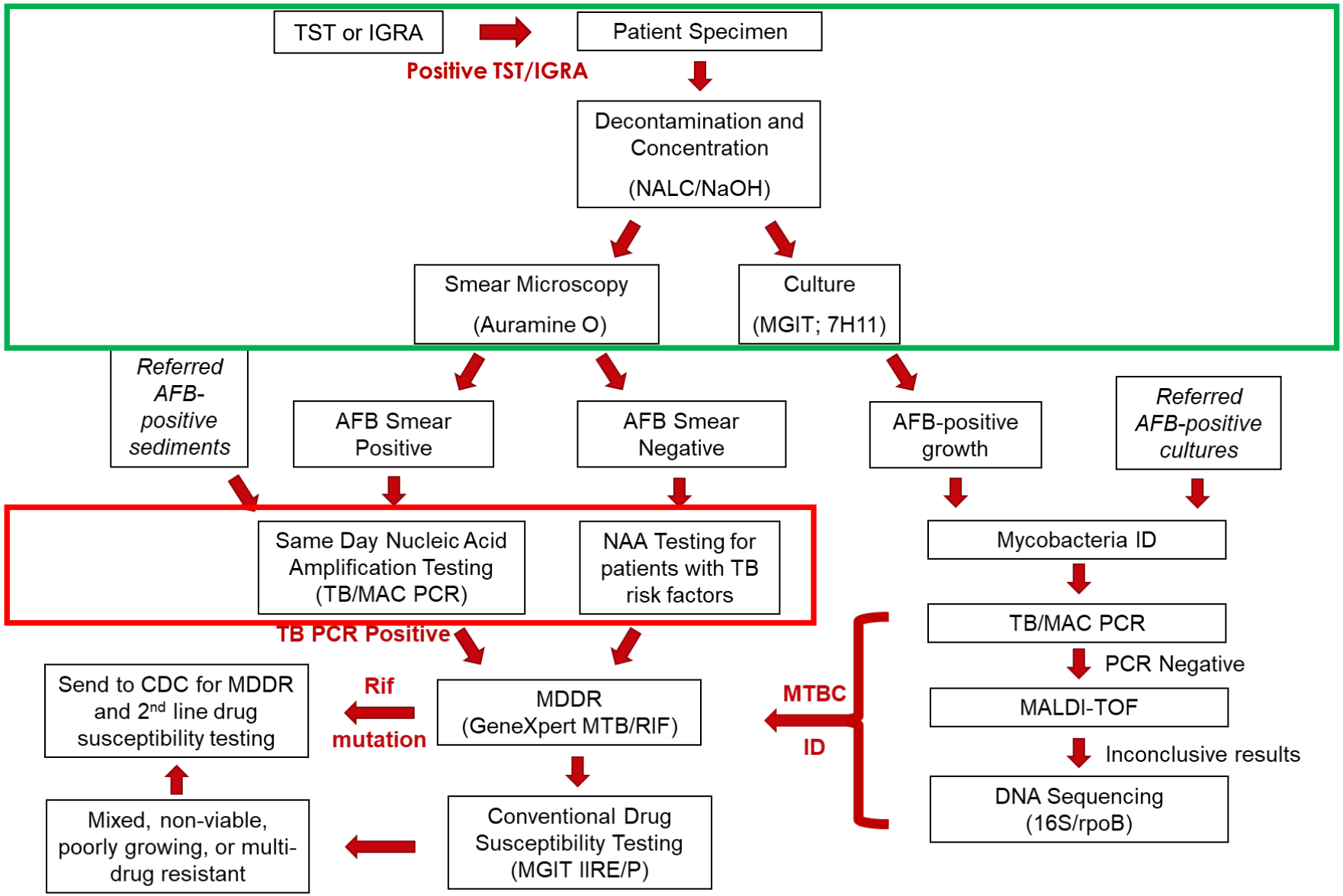
Specimen receipt
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14+

Can we speed this process up?

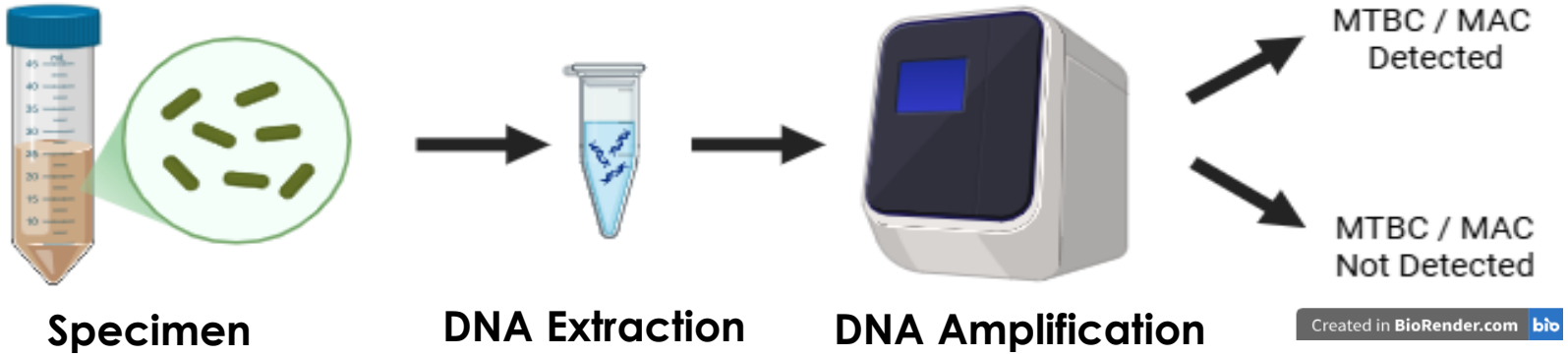
Modern Mycobacteriology

Rapid screen for *potential* MTBC infection

Rapid ID of MTBC from primary specimens



Rapid ID of MTBC from Primary Specimens



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

- Molecular target: IS6110; present in multiple copies in most MTBC
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - ~50% of AFB smear-negative, 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

WSLH TB PCR: Fee-exempt testing criteria

- Patient must have signs and symptoms of pulmonary TB
- Patient must be reported to the local or state public health department as a suspect TB case as required by Wisconsin Statute Chapter 252.05 and Wisconsin Administrative Code Chapter HFS 145.04 (3)(a)
- Patient must be in respiratory isolation (for pulmonary disease)

- Patient must not have been diagnosed with TB or a non-tuberculous mycobacterial infection within the last 12 months
- Patient must have received ≤7 days of anti-mycobacterial therapy or no such treatment within the last 12 months

If patient is TB suspect, smear-negative PCR can be run with WI TB Program approval

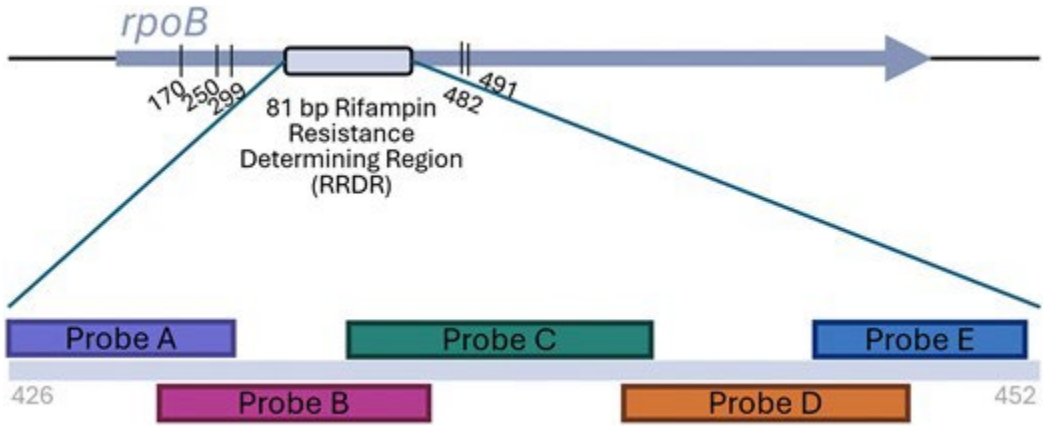
- **Contact WI TBP → Will send approval to WSLH**

Rapid ID of MTBC from Primary Specimens

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

Cepheid



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

Interpreting GeneXpert MTB/RIF results

3 potential results based on the absence/presence of *rpoB* mutations

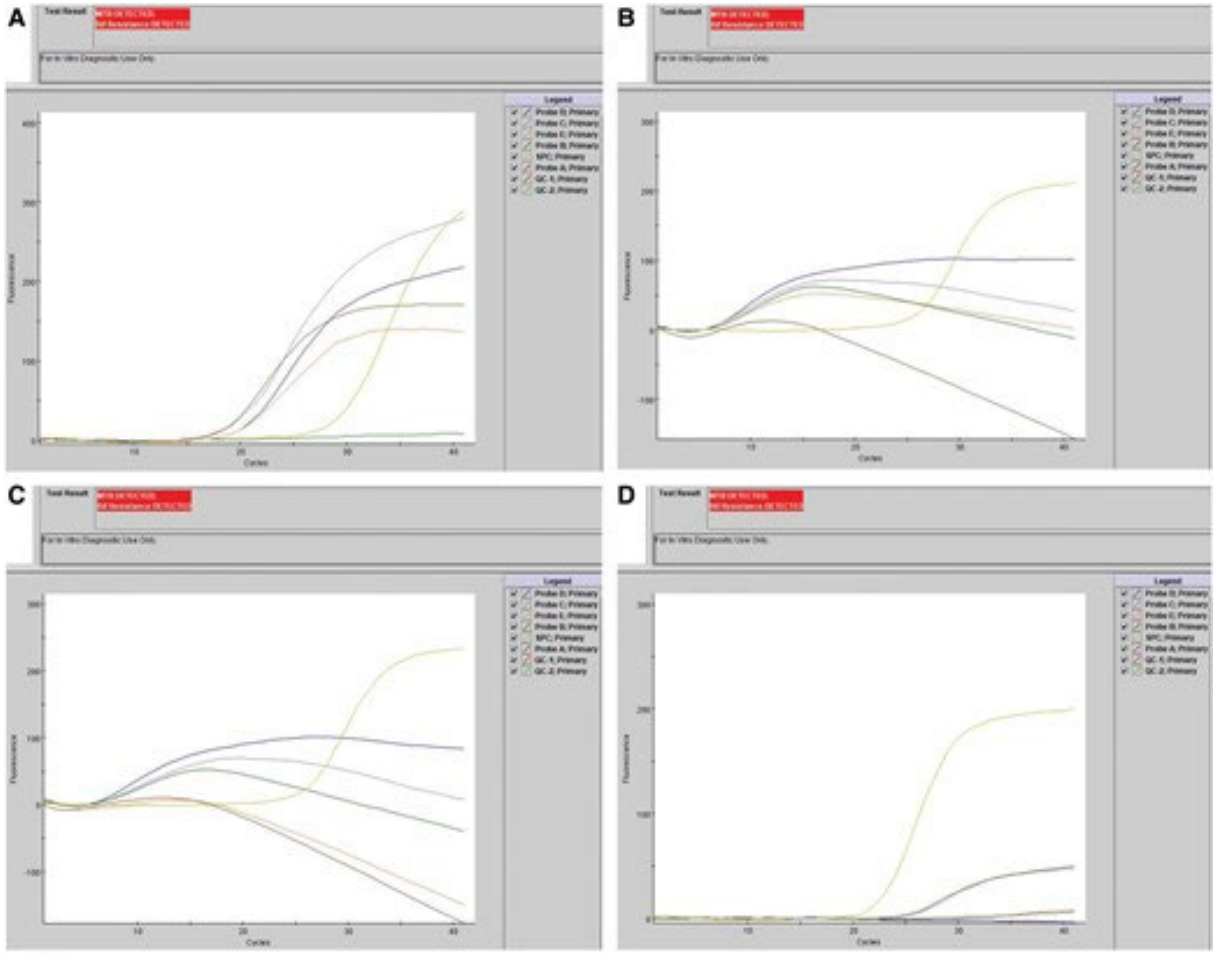
- MTB Not Detected → No MTB, or level of MTB is below limit of detection (ie: AFB smear-negative specimens)

- MTB Detected, No RIF-resistance Detected
  - No mutations detected, patient MTB likely susceptible to RIF
  - Some RIF-resistance mutations outside target area, so still need phenotypic susceptibilities!

- MTB Detected, RIF-resistance Detected
  - *rpoB* mutation detected, isolate potentially RIF-resistant
    - Could be any mutation, not specifically one conferring RIF-resistance
  - Needs to be confirmed by either DNA sequencing or phenotypic testing (or both)!

Be aware of potential for false-positive and false-negative results!

- Known issues:
  - False-positive MTBC
    - Previous TB patients may remain NAAT positive for >1 year
    - Possible cross-reactivity with rare NTM species, or NTM at very high CFU/ml
  - False-positive RIF-resistance
    - Silent mutations in probes A,B,D,E
    - Very high MTB CFU/ml
  - False-negative RIF-resistance
    - *rpoB* mutations outside RRDR
- Check your curves!
- If RIF-resistance is predicted, send to WSLH for additional testing!



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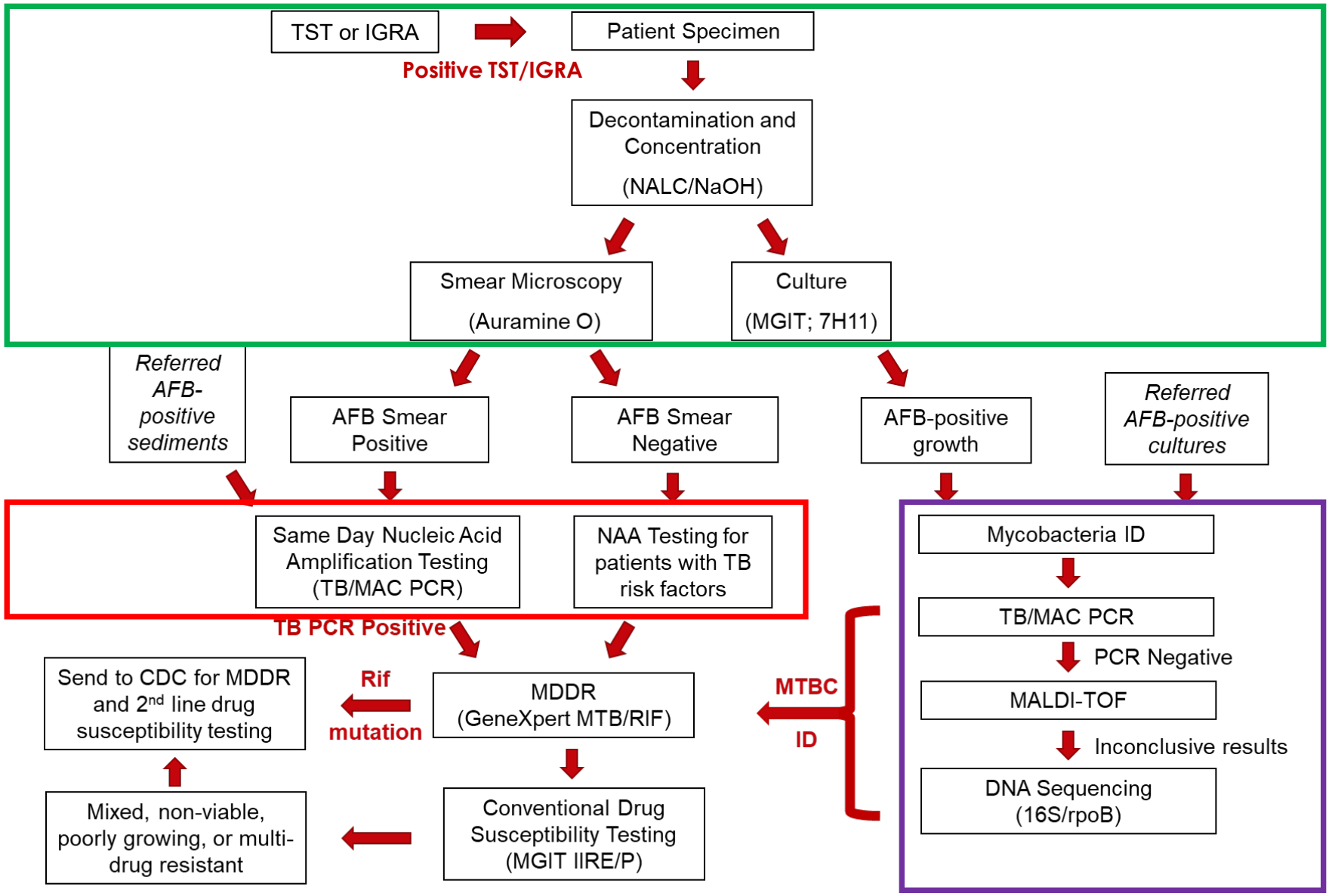
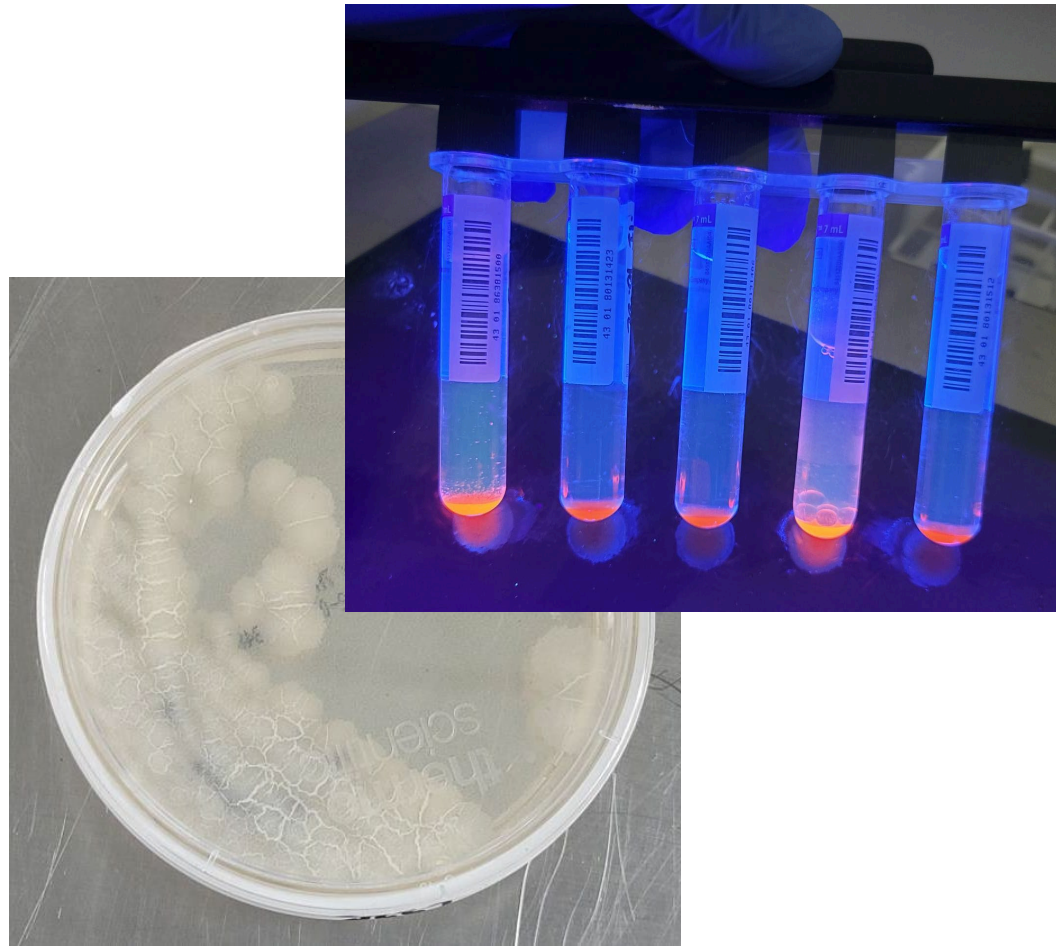


ID of MTBC from positive culture

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

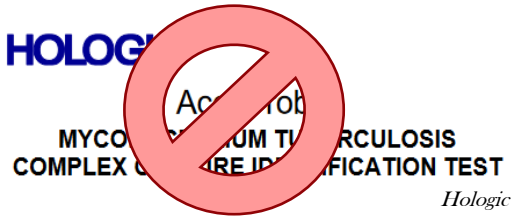
- "Gold standard"
  - Most sensitive method for mycobacterial detection (10 viable AFB/ml)
- Slowest method
  - Average time to detection: 15 days
  - Range: 4-40+ days
- Culture required for certain testing
  - Phenotypic DST
  - Genotyping



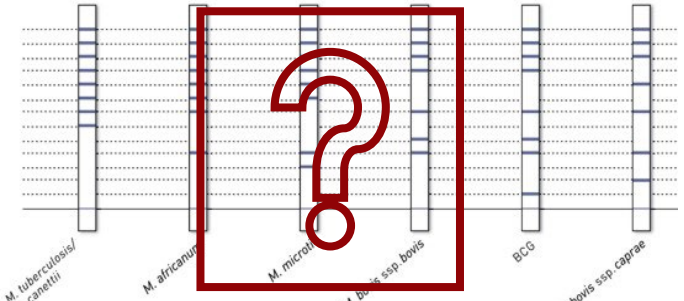
ID of MTBC from positive culture

Cepheid

Bruker



Hologic



ThermoFisher

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

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

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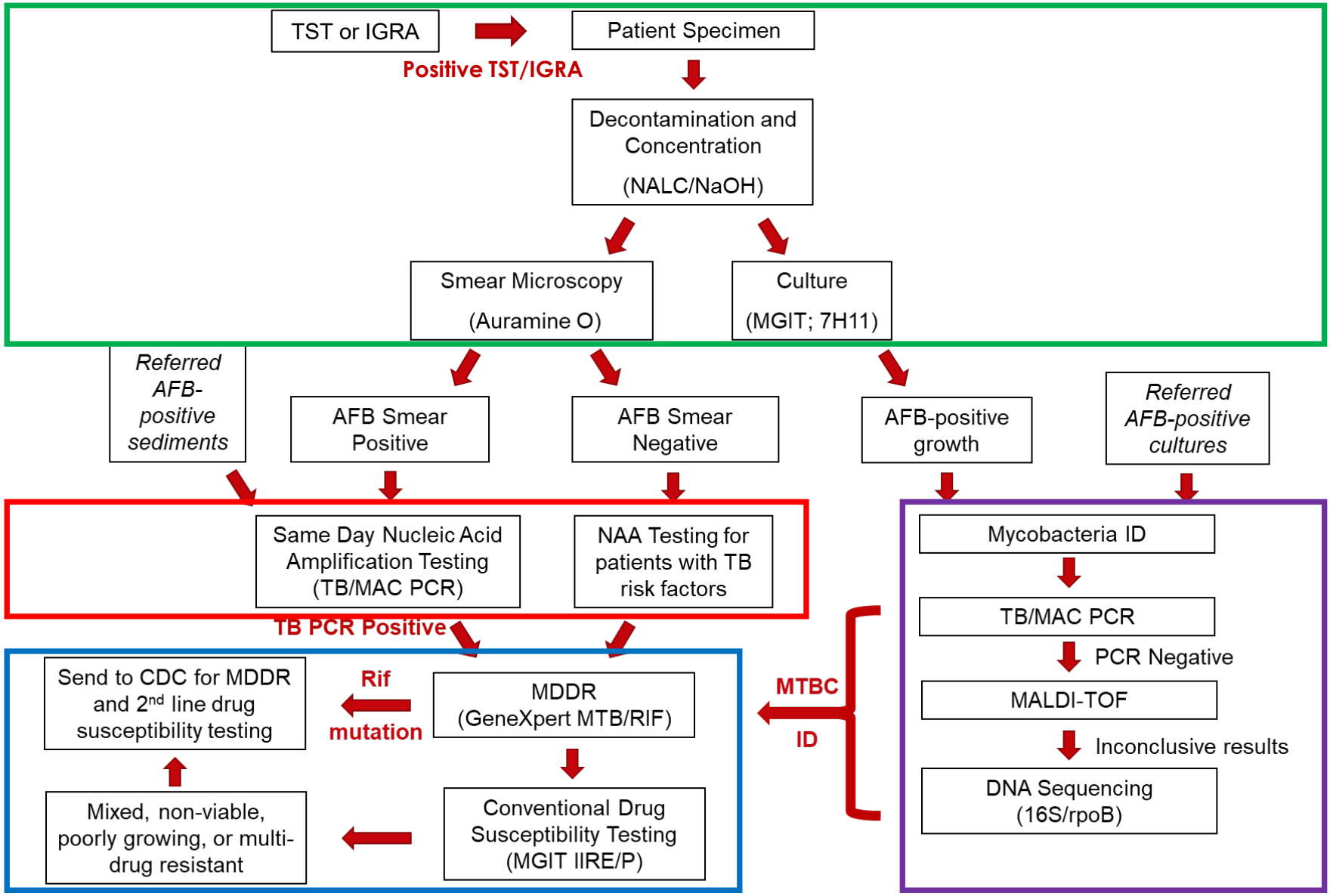
Access to MTBC drug susceptibility testing



MTBC Drug Susceptibility Testing (DST)

- 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

RESEARCH

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

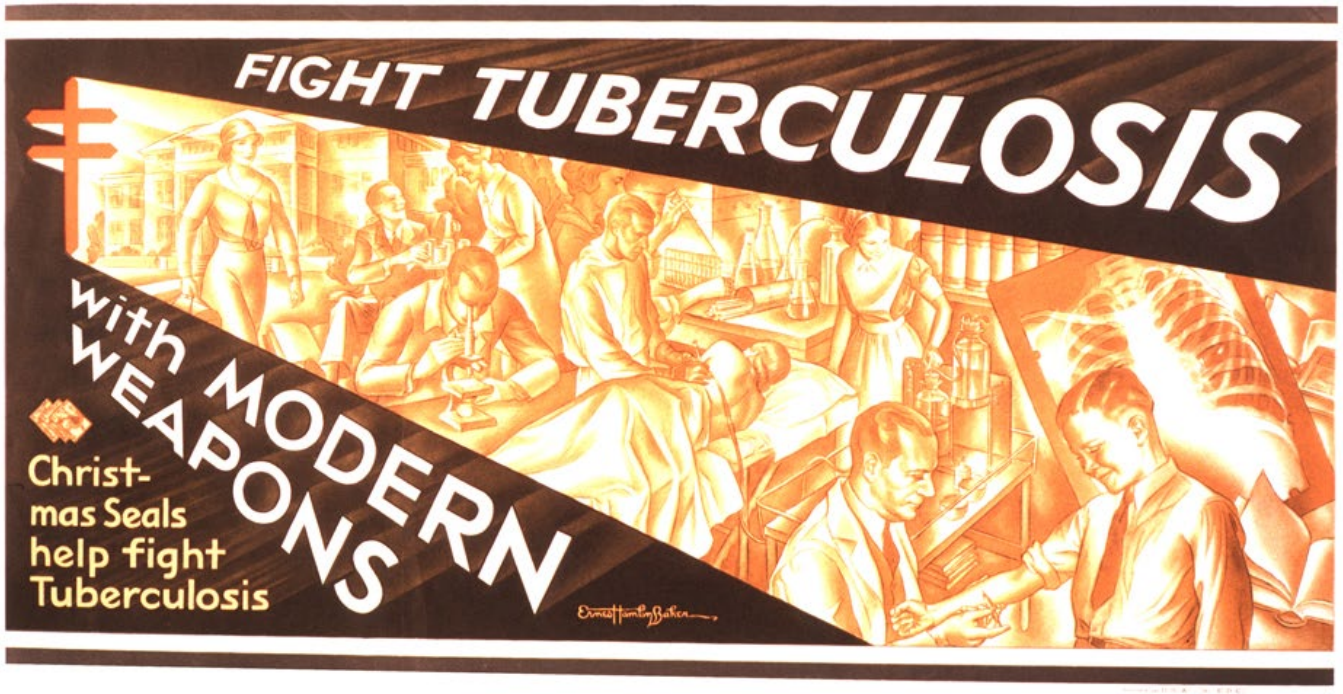
DISPATCHES

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

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

Standard First Line Therapy (RIPE/HRZE regimen)

- **R**ifampin (RIF/R)
  - Inhibits RNA polymerase (rpoB)
  - Incredible penetration, even to CNS
- **I**soniazid (INH/H)
  - Prodrug (katG)
  - Inhibits mycolic acid synthesis
- **P**yrazimamide (PZA/Z)
  - Prodrug (pncA); MOA= ?
  - Active at lower pH
- **E**thambutol (EMB/E)
  - Inhibits cell wall synthesis



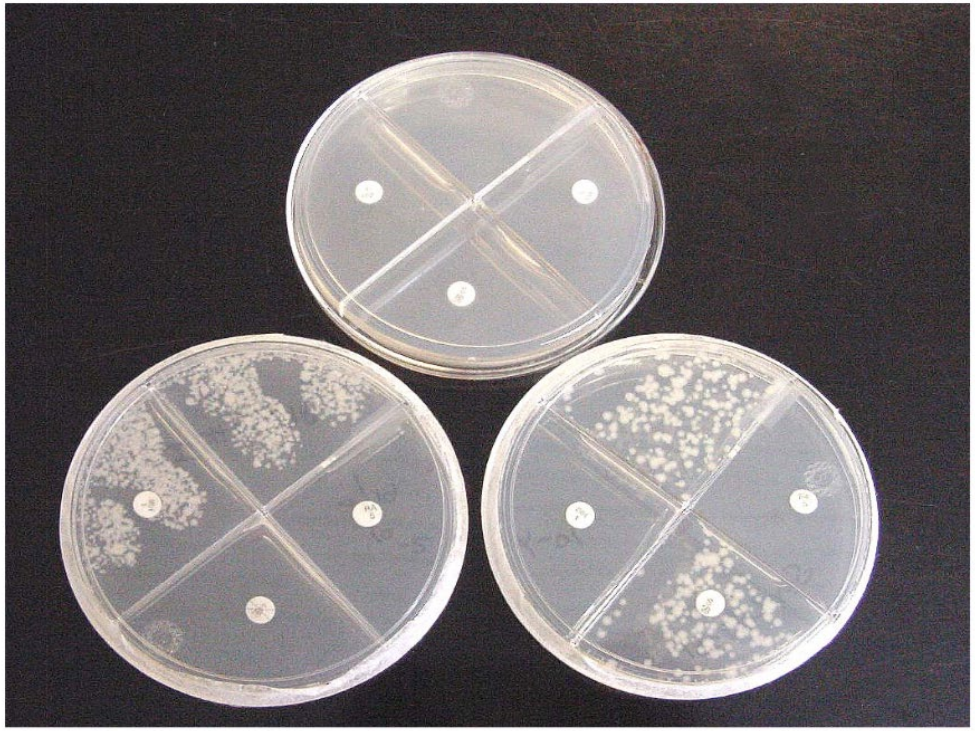
MTBC Drug Susceptibility Testing: Phenotypic

BD MGIT, Versatrek, Sensititre MTB, agar proportion

Phenotypic AST: Incubate a standardized concentration of MTBC isolate with a known concentration of a drug

- 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

And then, several eternities pass (relatively speaking)

Positive culture
(2+ weeks)

Positive AFB Smear
(<24 hours)

Specimen receipt
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14+

TB PCR **ID** for
smear positives
(<1 day)

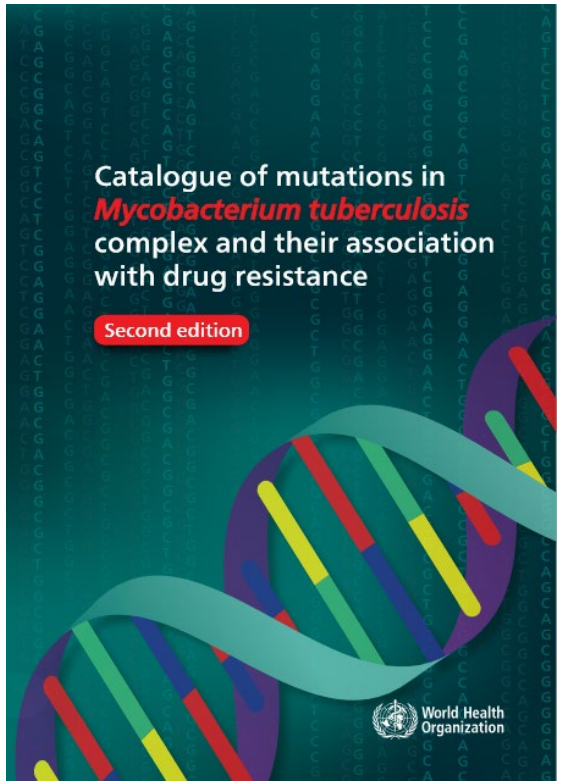
MTBC Drug Susceptibility Testing: Molecular

GeneXpert, next-generation sequencing (NGS)

- 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
  - Unknown or unlinked mutations
  - Higher level sequencing requires bioinformatics know-how

Molecular DST: Examine the DNA of MTBC isolate

- Are there mutations present known or suspected to result in drug resistance?

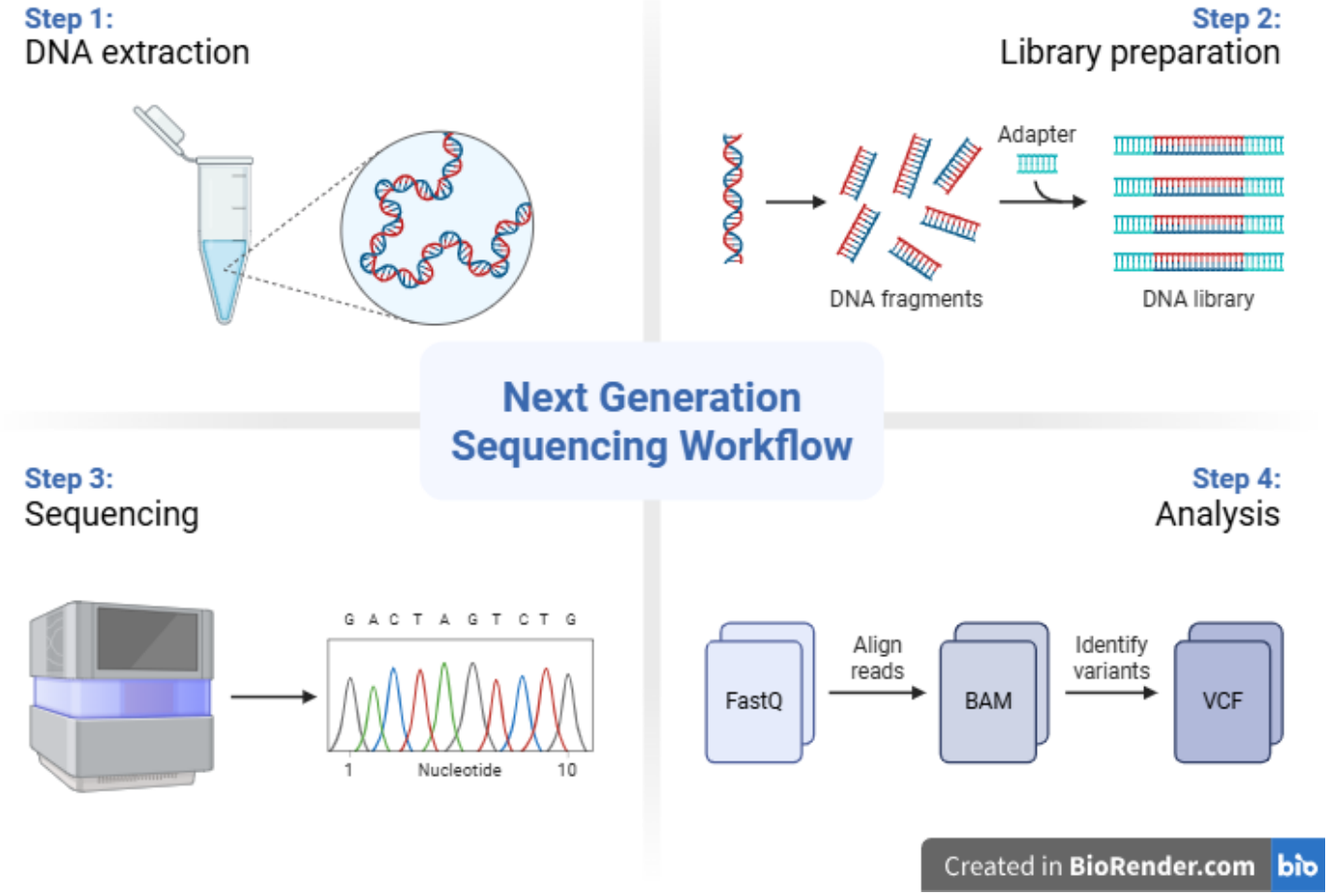


MTBC Drug Susceptibility Testing: Molecular

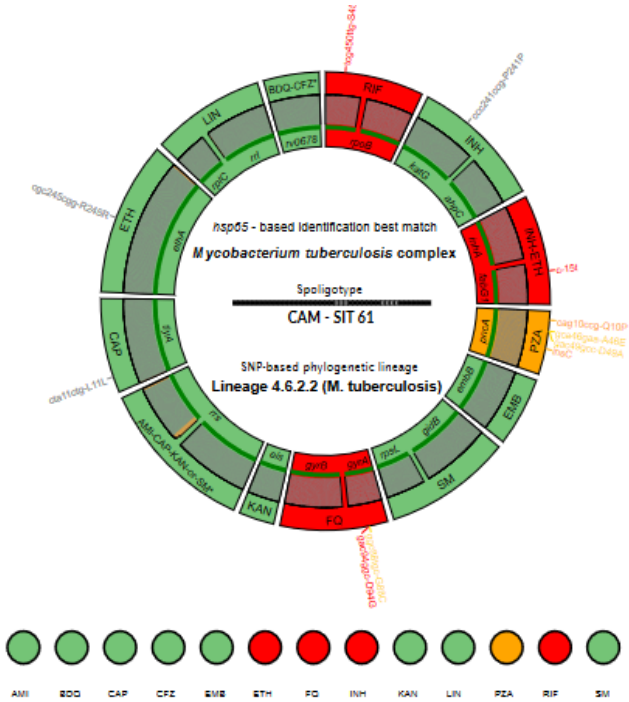
Next-generation sequencing (NGS)

- 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



MTBC Drug Susceptibility Testing: tNGS



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

MTBC tNGS kits

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

- Deeplex Myc-TB
  - Amplifies 18 MTBC resistance genes, ID, spoligotype

CDC Molecular Detection of Drug Resistance Program

- 15 gene targets covering 11+ drugs
  - RIF, INH, EMB, PZA, FQ, AMK, CAP, KAN, BDQ, CLF, LZD
- Molecular data confirmed with phenotypic testing for majority of drugs

Pre-approval required for all testing

MTBC Agar Proportion Susceptibility*	% Resistant	Interpretation
Isoniazid 0.2 µg/mL	100 %	Resistant
Isoniazid 1.0 µg/mL	100 %	Resistant
Isoniazid 5.0 µg/mL	67 %	Resistant
Rifampin 1.0 µg/mL	0 %	Susceptible
Ethambutol 5.0 µg/mL	33 %	Resistant
Streptomycin 2.0 µg/mL	100 %	Resistant
Streptomycin 10.0 µg/mL	33 %	Resistant
Rifabutin 2.0 µg/mL	0 %	Susceptible
Ciprofloxacin 2.0 µg/mL	0 %	Susceptible
Kanamycin 5.0 µg/mL	0 %	Susceptible
Ethionamide 10.0 µg/mL	0 %	Susceptible
Capreomycin 10.0 µg/mL	0 %	Susceptible
PAS 2.0 µg/mL	0 %	Susceptible
Ofloxacin 2.0 µg/mL	0 %	Susceptible
Amikacin 4.0 µg/mL	0 %	Susceptible

MTBC Drug Susceptibility Testing: WGS

```
Taxonomic report:

Unclassified reads:      0.09%

Mycobacterium tuberculosis complex       99.89%
Mycobacterium bovis      7.77%
Mycobacterium bovis BCG 2.72%

RD1 region absent, likely Mycobacterium bovis BCG


Mycobacterium Species detected : Mycobacterium bovis BCG
```

```
Mapping Statistics (Downsampled ratio 0.980630565600288) :

Total # of mapped reads :        2240834
Percent of mapped reads:         99.40%
Genome Coverage =        0.969308
Average Depth =  111.326

# Homozygous positions = 4213184
# Heterozygous positions = 331
Percentage of Heterozygous Positions: 0.0079%
```

```
rpoB: PASS
katG: PASS
furA-katG promoter region: PASS
oxyR-ahpC promoter region: PASS
inhA: PASS
mabA-inhA promoter region: PASS
mabA: PASS
pncA: PASS
pncA promoter region: PASS
embB: PASS
embC-embA promoter region: PASS
rpsL: PASS
rrs 512, 513, 516, 906: PASS
rrs 1400: PASS
eis promoter region: PASS
gyrA: PASS
gyrB: PASS
ethA: PASS
panD: PASS
rrl: PASS
rplC: PASS
ndh: PASS
gidB: PASS
fbiD: PASS
fbiA: PASS
fbiB: PASS
fbiC: PASS
Rv0678: PASS
atpE: PASS
pepQ: PASS
```

```
All Mutations in screened loci:

5750    171     GTG -> GTA      Val -> Val      Rv0005  gyrB    Fluoroquinolones        1/1 (Pure) (Silent)
6404    389     AAC -> AAT      Asn -> Asn      Rv0005  gyrB    Fluoroquinolones        1/1 (Pure) (Silent)
6446    403     GCG -> TCG      Ala -> Ser      Rv0005  gyrB    Fluoroquinolones        1/1 (Pure) (Neutral)
7362    21      GAG -> CAG      Glu -> Gln      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Neutral)
7584    95      AGC -> ACC      Ser -> Thr      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Neutral)
8283    328     ATC -> ATT      Ile -> Ile      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Silent)
8622    441     CGG -> CGT      Arg -> Arg      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Silent)
8739    480     CGC -> CGT      Arg -> Arg      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Silent)
9141    614     ATT -> ATC      Ile -> Ile      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Silent)
9303    668     GGC -> GAC      Gly -> Asp      Rv0006  gyrA    Fluoroquinolones        1/1 (Pure) (Neutral)
491740  320     TTT -> TTC      Phe -> Phe      Rv0407  fgd1    Delamanid/Pretomanid    1/1 (Pure) (Silent)
763029  1075    GCT -> GCC      Ala -> Ala      Rv0667  rpoB    Rifampin        1/1 (Pure) (Silent)
763574  69      CGC -> CCC      Arg -> Pro      Rv0668  rpoC    Rifampin compensatory   1/1 (Pure) (Neutral)
776101  794     ACC -> ATC      Thr -> Ile      Rv0676c mmpL5   Clofazimine/Bedaquiline 1/1 (Pure) (Neutral)
775639  948     ATT -> GTT      Ile -> Val      Rv0676c mmpL5   Clofazimine/Bedaquiline 1/1 (Pure) (Neutral)
781566  3       ACC -> ACT      Thr -> Thr      Rv0682  rpsL    Streptomycin    1/1 (Pure) (Silent)
1417554 -207    C -> G          intergenic Rv1268c-embR Rv1268c-embR promoter region    Ethambutol      1/1 (Pure) (Neutral)
1917970 11      CTA -> CTG      Leu -> Leu      Rv1694  tlyA    Aminoglycosides 1/1 (Pure) (Silent)
2102106 313     GGG -> CGG      Gly -> Arg      Rv1854c ndh     Isoniazid       1/1 (Pure) (Neutral)
2103174 -131    Intergenic deletion     -G      intergenic ndh  ndh promoter region     Isoniazid       1/1 (Pure) (Unknown)
2156027 29      CCC -> CCA      Pro -> Pro      Rv1908c katG    Isoniazid       1/1 (Pure) (Silent)
2155505 203     ACC -> ACT      Thr -> Thr      Rv1908c katG    Isoniazid       1/1 (Pure) (Silent)
2154725 463     CGG -> CTG      Arg -> Leu      Rv1908c katG    Isoniazid       1/1 (Pure) (Neutral)
2156466 43      GCC -> GTC      Ala -> Val      Rv1909c furA    Isoniazid       1/1 (Pure) (Neutral)
2289073 57      CAC -> GAC      His -> Asp      Rv2043C pncA    Pyrazinamide    1/1 (Pure) (HC mutation)
3336825 365     ACA -> GCA      Thr -> Ala      Rv2981c ddlA    D-Cycloserine   1/1 (Pure) (Neutral)
4038403 768     TTG -> CTG      Leu -> Leu      Rv3596c clpC1   Pyrazinamide    1/1 (Pure) (Silent)
4240670 270     ACC -> ATC      Thr -> Ile      Rc3793  embC    Ethambutol      1/1 (Pure) (Neutral)
4242029 723     CTG -> TTG      Leu -> Leu      Rc3793  embC    Ethambutol      1/1 (Pure) (Silent)
4242641 927     CGC -> CGT      Arg -> Arg      Rc3793  embC    Ethambutol      1/1 (Pure) (Silent)
4242968 1036    ACC -> ACT      Thr -> Thr      Rc3793  embC    Ethambutol      1/1 (Pure) (Silent)
4244220 330     CTG -> TTG      Leu -> Leu      Rv3794  embA    Ethambutol      1/1 (Pure) (Silent)
4246550 13      AAT -> AGT      Asn -> Ser      Rv3795  embB    Ethambutol      1/1 (Pure) (Neutral)
4246862 117     GTC -> GTT      Val -> Val      Rv3795  embB    Ethambutol      1/1 (Pure) (Silent)
4247171 220     CTG -> CTA      Leu -> Leu      Rv3795  embB    Ethambutol      1/1 (Pure) (Silent)
4247645 378     GAG -> GCG      Glu -> Ala      Rv3795  embB    Ethambutol      1/1 (Pure) (Neutral)
4407590 205     GCA -> GCG      Ala -> Ala      Rv3919c gidB    Streptomycin    1/1 (Pure) (Silent)


Number of mutation(s) found in screened loci = 37
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CLIMS SECTION:

Sample ID       WI257
Analysis Date   12/21/2023

Test    Property        Notation        Result
WGS_HI_CONF_MUTATION    rpoB    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    katG    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    oxyR-ahpC promoter region       PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    inhA    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    mabA-inhA promoter region       PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    mabA    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    pncA    PASS    His57Asp
WGS_HI_CONF_MUTATION    pncA promoter region    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    embB    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    embC-embA promoter region       PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    rpsL    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    rrs 512, 513, 516, 906  PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    rrs 1400        PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    eis promoter region     PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    gyrA    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    gyrB    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    ethA    PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    rrl     PASS    No high-confidence mutation
WGS_HI_CONF_MUTATION    rplC    PASS    No high-confidence mutation

WGS_TB_RESISTANCE       Rifampin        PASS    Susceptible
WGS_TB_RESISTANCE       Isoniazid       PASS    Susceptible
WGS_TB_RESISTANCE       Pyrazinamide    PASS    RESISTANT (predicted)
WGS_TB_RESISTANCE       Ethambutol      PASS    Susceptible
WGS_TB_RESISTANCE       Streptomycin    PASS    Susceptible
WGS_TB_RESISTANCE       Kanamycin/Amikacin      PASS    Susceptible
WGS_TB_RESISTANCE       Fluoroquinolones        PASS    Susceptible
WGS_TB_RESISTANCE       Ethionamid      PASS    Susceptible
WGS_TB_RESISTANCE       Linezolid       PASS    Susceptible
WGS_TB_RESISTANCE       Delamanid/Pretomanid    PASS    Susceptible
WGS_TB_RESISTANCE       Clofazimine/Bedaquiline PASS    Susceptible
WGS_TB_RESISTANCE       Bedaquiline     PASS    Susceptible
```

MTBC Drug Susceptibility Testing: WGS

- WSLH has sequenced close to 400 MTBC isolates over the past 5 years
- Validation focused on MTBC species ID, 1st-line drug susceptibility prediction (INH, RIF, EMB, PZA)
- Validation set included:
  - *M. tuberculosis, M. bovis, M. bovis*-BCG, and common non-tuberculous mycobacteria
  - Isolates mono-resistant to INH, RIF, PZA, MDR-TB, pan-resistant strains, pan-susceptible strains
- Initially validated on MiSeq, NextSeq2000 → Completing validation of MiSeq i100
- Initially validated using APHL-hosted pipeline→ Validating in-house analysis pipeline

MTBC WGS Validation Summary

MTBC Identification: Accuracy

M. tuberculosis Identification	*M. bovis* Identification	*M. bovis*-BCG Identification	Non-MTBC identification
100%	100%	100%	100%

Did WGS correctly predict species?

Phenotypic DST: Accuracy

	Rifampin	Isoniazid	Ethambutol	Pyrazinamide
Positive Prediction	100%	88.2%†	100%	84.2%‡
Negative Prediction	95.6%	100%	100%	97.22%

Did WGS correctly predict resistance?

Did WGS correctly predict susceptibility?

Genotypic DST: Accuracy

	Rifampin	Isoniazid	Ethambutol	Pyrazinamide
Positive Prediction	100%	100%	100%	100%

Did WGS correctly identify known mutation?

†: 2 isolates expected to be INH resistant had *katG*(D94N) mutation of unknown significance
‡: 3 isolates expected to be PZA resistant with *ClpC1*(V63A) disputed mutation

WSLH: TB Whole Genome Sequencing Reports

	Collected	Type	Source	Submitter ID
26MM000026	4/2/2026 1500	Sputum		

M. tuberculosis complex WGS (Final result)

ID: 26MM000026 Type/Src: Sputum

	Result	Units
MTBC Identification	Mycobacterium tuberculosis	
Isoniazid DST Prediction	No high-confidence mutations associated with resistance to INH have been detected.	
Rifampin DST Prediction	Mutation(s) associated with resistance to RIF detected.	!
Ethambutol DST Prediction	Mutations with unknown significance have been detected. Resistance to EMB cannot be ruled out.	!
Pyrazinamide DST Prediction	No Result - Unable to amplify target sequence	

Comments:
This next-generation sequencing-based test is designed for the identification and prediction of drug susceptibility in Mycobacterium tuberculosis complex (MTBC) isolates grown in MGIT broth. This test was developed and its performance characteristics determined by the Wisconsin State Laboratory of Hygiene, a Clinical Laboratory Improvement Amendments (CLIA) certified, high complexity clinical laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration.

This sequence-based prediction of drug susceptibility only identifies known mutations in common resistance-linked MTBC genetic loci. Novel mutations or potential contributions to drug resistance outside these regions will not be detected by this assay.

Phenotypic susceptibility testing is in progress.

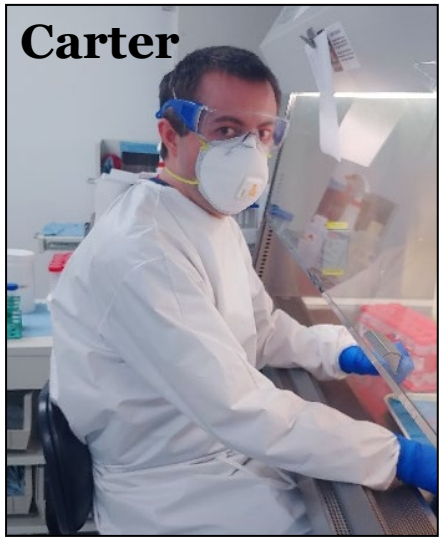
WSLH: TB DST Whole Genome Sequencing Notes

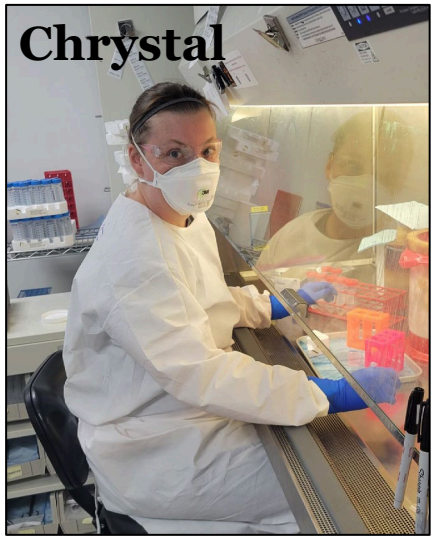
- Testing is performed on pure MTBC cultures only
  - tNGS for primary specimens will not be validated at this time
- Mutations associated with resistance suggest the patient isolate will not respond favorably to use of that drug → Adjust therapeutic regimen more rapidly
- Phenotypic testing (except PZA) will be performed on all isolates, regardless of WGS results for the foreseeable future
  - TB WGS will be replacing phenotypic PZA testing due to repeated manufacturer QC issues
- Specimens for which a single drug fails will not be repeated unless phenotypic testing for that isolate fails
- Detection of high-confidence resistance mutations that confer low-level drug resistance may result in discordant genotypic and phenotypic susceptibility results
- WSLH plans to expand validation to include reporting of additional drugs in the future!

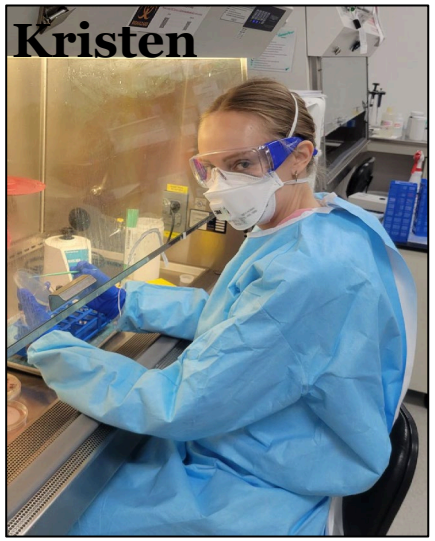
MTBC Diagnostics: So, What Do You Know Now?

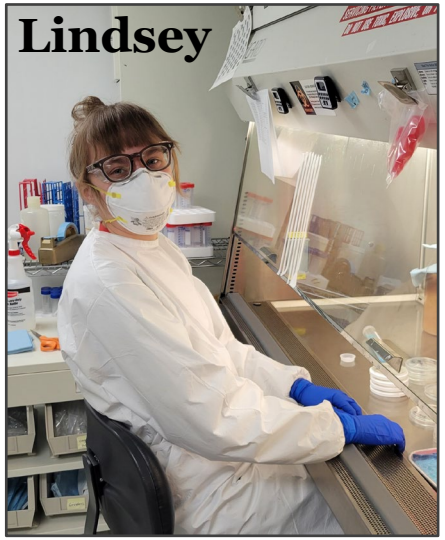
- Large portion of MTBC diagnostic testing is unchanged since the 1900s
  - MTBC culture is still the king!
- Accessing new MTBC testing is becoming increasingly difficult for US laboratories
  - Many new platforms or tests not available in US
  - Reagents have become increasingly centralized into just a few companies
  - 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

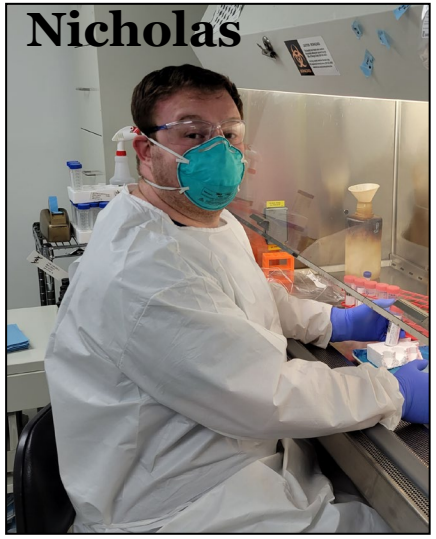
WSLH TB Laboratory Team











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