“I would like to order an MDDR with a side of MIC and WGS. What is your traditional DST today?”

Benjamin Alpers & Jan Owen

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New Directions in TB
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Benjamin Alpers has the following disclosures to make:

- No conflict of interests
- No relevant financial relationships with any commercial companies pertaining to this educational activity

Jan Owen has the following disclosures to make:

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TEXAS
Health and Human Services

Texas Department of State Health Services

“I would like to order an MDDR with a side of MIC and WGS. What is your traditional DST today?”

or

Making Sense of the TB Diagnostic Menu

Benjamin Alpers, Applications Scientist/TB Reference Team Lead

Jan Owen, Mycobacteriology/Mycology Branch Manager

DSHS Austin Laboratory

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TEXAS Health and Human Services

Texas Department of State Health Services

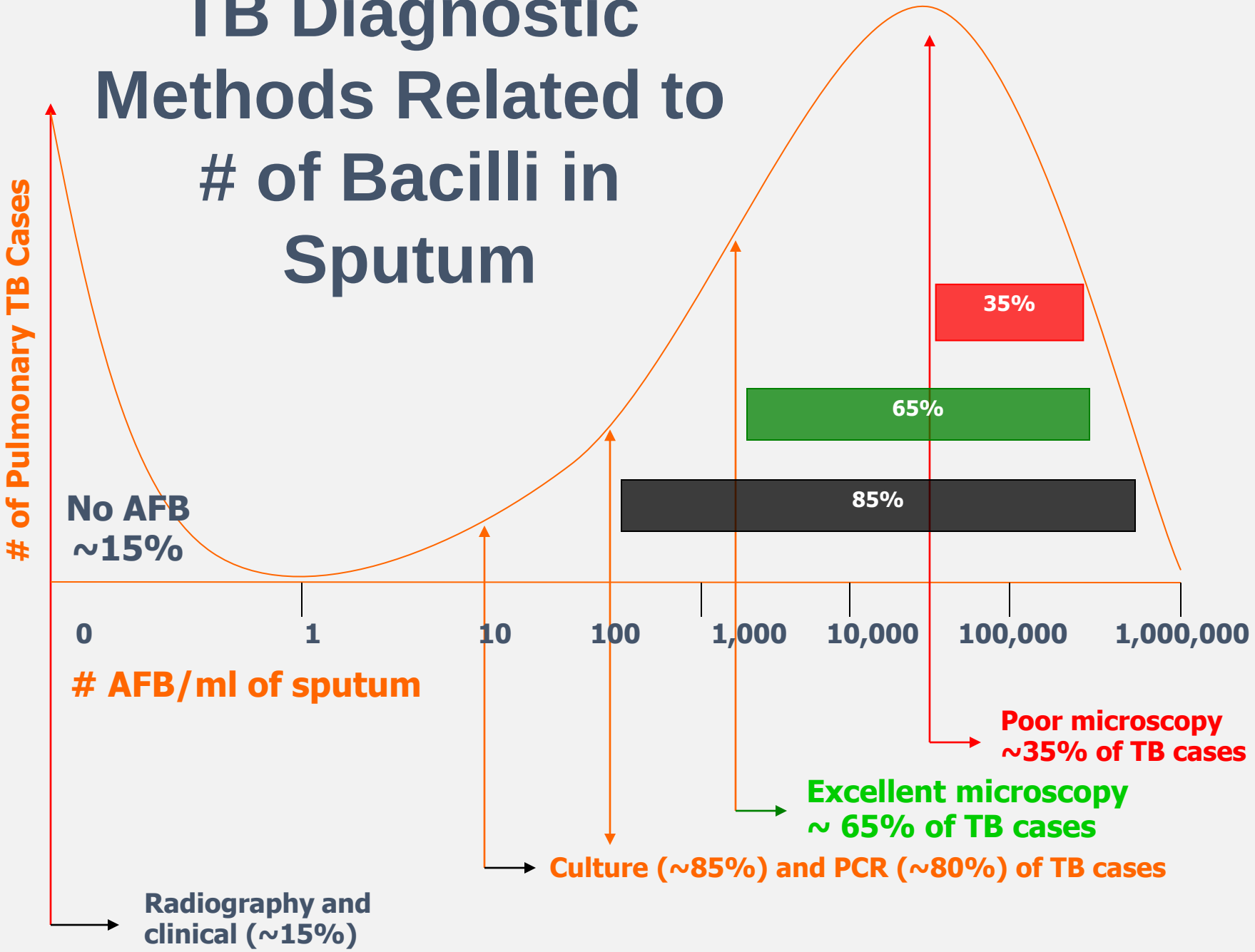
AFB Smear

- Used in diagnosis for 100+ years
- Rapid and universally available
    - We strive for 24hr TAT
- Identifies priority specimens for NAAT
    - 5,000 to 10,000 AFB/ml must be present in specimen to be detected in smear
- Not sensitive
    - Misses ~50% of TB
- Not specific
    - Positive smear may be NTM
- Can detect dead bacilli

AFB Culture

- More sensitive than smear
    - ~10 viable AFB/ml for culture
- Positive for ~85% of Pulmonary TB
    - Requires a quality specimen
    - May be invalid due to contamination
- Used to monitor patient response to treatment (like smear)
- Required for conventional drug susceptibilities & genotype
- Lengthy
    - 1-6 weeks by liquid media
    - 2-8 weeks by solid media

TB Diagnostic Methods Related to # of Bacilli in Sputum



Adapted from Priorities for TB Bacteriology Services in Low-Income Countries, 2007, IUATLD

Nucleic Acid Amplification Tests (NAAT)

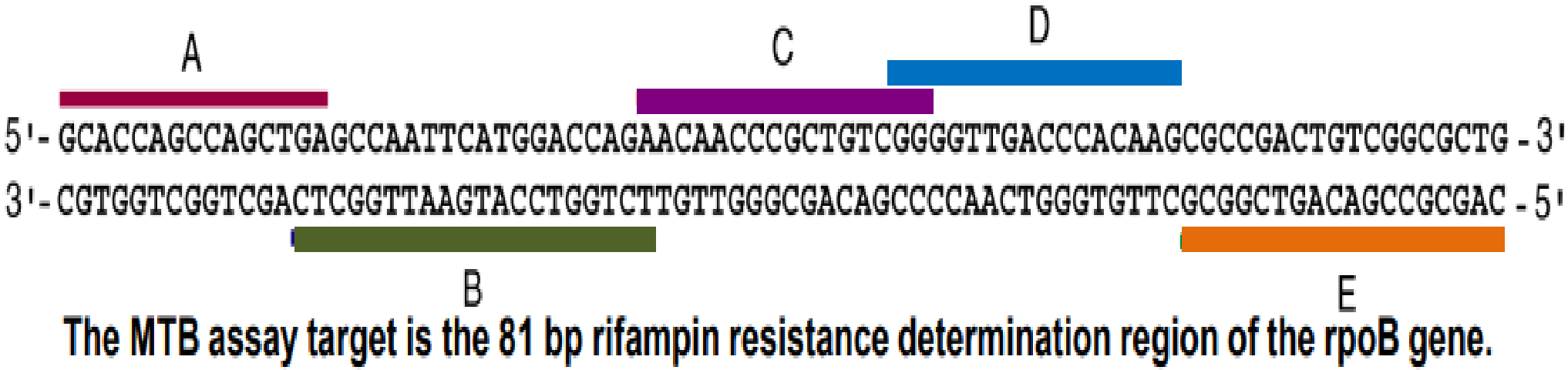
- Real time reverse transcription polymerase chain reaction (qRT-PCR or qPCR)
- Tiny amounts of DNA/RNA amplified (copied) until a significant signal compared to the background
- GeneXpert examines DNA for:
  - Identification
  - Detection of Rifampin Resistance
- Test turnaround time measured in hours

Nucleic Acid Amplification Tests (NAAT) cont.

- Detects *M. tuberculosis* complex nucleic acids
  - For initial diagnostic specimens only
  - Does not distinguish between live and dead bacilli
  - Not suitable for follow-up specimen or monitoring; cured patients may be NAAT + for years!

- Xpert sensitivity compared to TB culture
  - >95% for AFB smear-positive
  - Only 55-75% for AFB smear-negative

Cepheid GeneXpert® Target Region



The MTB assay target is the 81 bp rifampin resistance determination region of the rpoB gene.

Approx. 10% of rifampin resistant predictions are false (ex. Phe433Phe silent mutation)

GX rifampin resistant results must be confirmed by MDDR testing at the CDC.

Methods for Culture ID

- Lab-developed, species-specific PCR tests
- Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF or MALDI)
- High-performance liquid chromatography (HPLC)

Pro and Cons of ID Methods

- PCR highly sensitive but costly
  - Can only ID organism for which the assay was developed
- MALDI very specific
  - Can identify extremely wide range of organisms
  - Relatively poor sensitivity; requires substantial growth
- HPLC sensitive, inexpensive, and non-labor intensive
  - Chromatographs require highly trained interpretation
  - Often cannot distinguish organisms within complex or group

M. tb cx. Speciation through Real-Time Polymerase Chain Reaction (PCR)

Can differentiate between species within the complex using 5 sets of probes and primers targeting known regions of difference (RD)

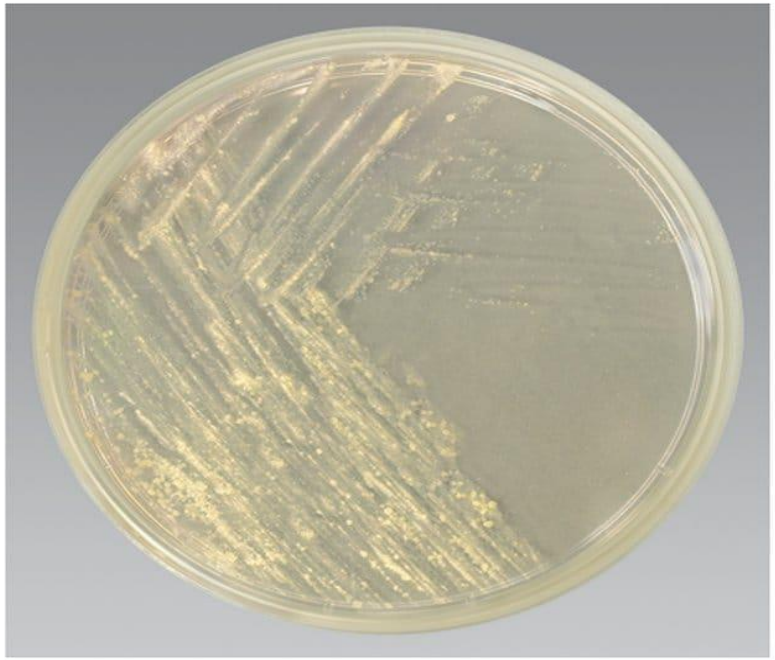
- *M. tuberculosis*
- *M. bovis*
- *M. bovis* BCG

} inherently PZA resistant

and

- *M. africanum*
- *M. microti*
- *M. canettii*
- *M. caprae*

…and others



TEXAS Health and Human Services

Texas Department of State Health Services

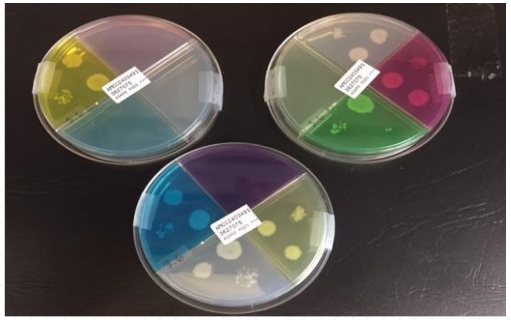
Conventional, Growth-based Drug Susceptibility Testing (DST)

MGIT 960 method

- Automated
- Widely available
- Poor accuracy
- Easily contaminated

Agar Proportion method

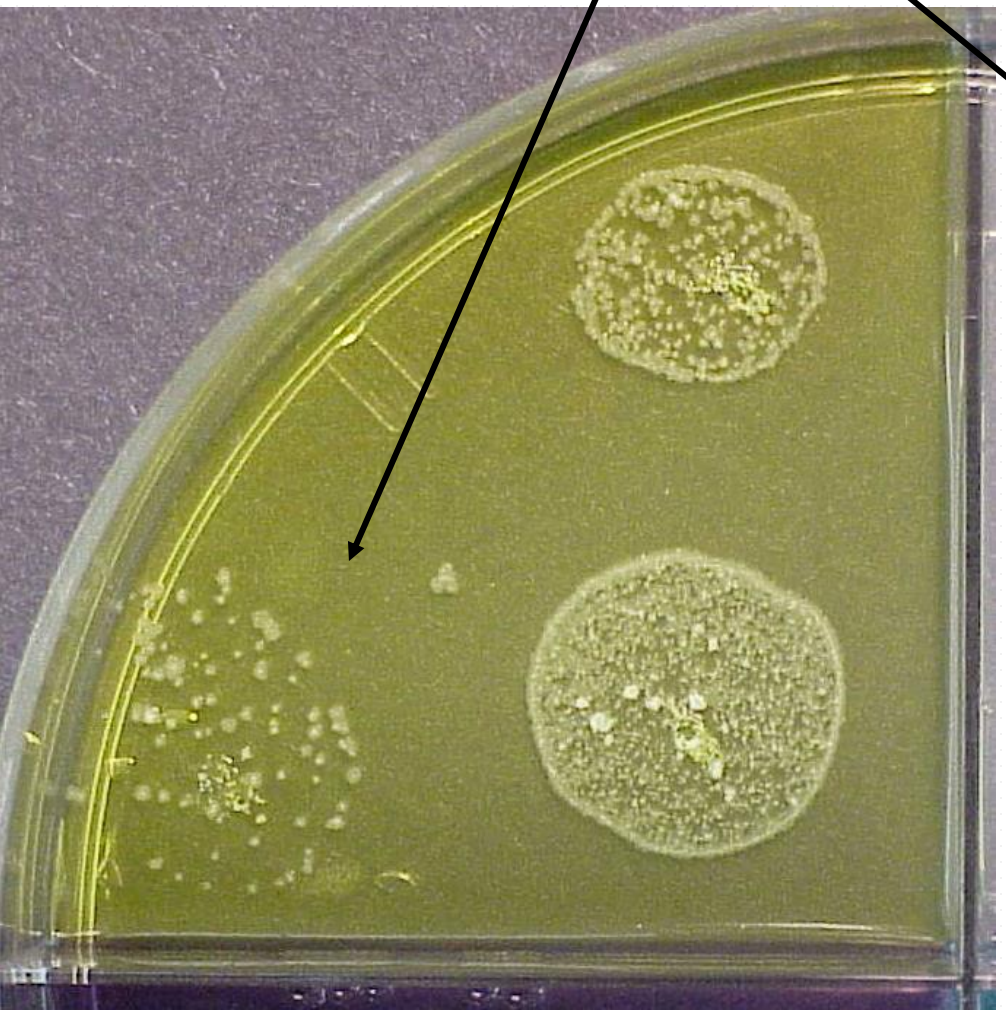
- Highly sensitive
- Inexpensive production
- Interpretation requires extensive experience



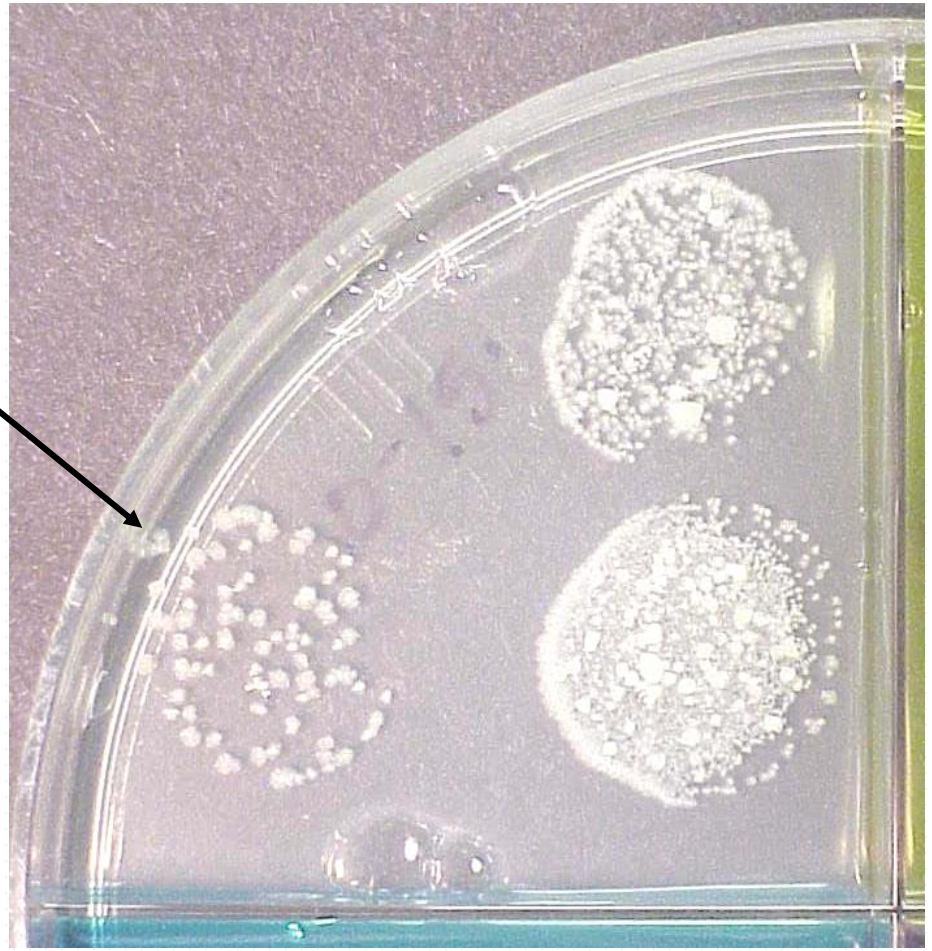
Agar Proportion (AP) DST Method Principle

- Standardized suspension inoculated to quadrant plates
  - Drug-containing Middlebrook 7H10 agar
  - Drug-free control
- Drug no longer counted on as being effective if:
  - Growth of on drug quadrant is 1% or greater than the growth on the control

100cfu/100cfu = 100% Resistance



Isoniazid, 1.0 mcg/ml



Drug-Free Control

Additional Considerations

- CDC recommends RMP DST results be reported within 17 days after *M. tuberculosis* culture identification
- Most U.S. laboratories use a rapid commercial system for DST (MGIT 960)
- Commercial DST methods miss some clinically significant RMP resistance that can be detected by agar proportion
- Agar Proportion (AP) is the “gold standard” method for conventional DST…however AP is not a rapid method; conventional AP method takes 21 days (3 weeks) for full results
- DSHS regularly reports INH & RMP susceptibility within 17 days of culture identification
- PZA testing can only be performed by MGIT 960 method

Minimum Inhibitory Concentration (MIC)

- Lowest concentration of a drug which prevents detectable in vitro growth when tested in a series of concentrations
- Only available at specialized laboratories such as CAHD and Wadsworth Center NYHD
- Performed only by special request on select antibiotics
- Tested by MGIT, AP, or Broth Micro-dilution (BMD)

Types of Molecular Sequencing

Required DNA Volume

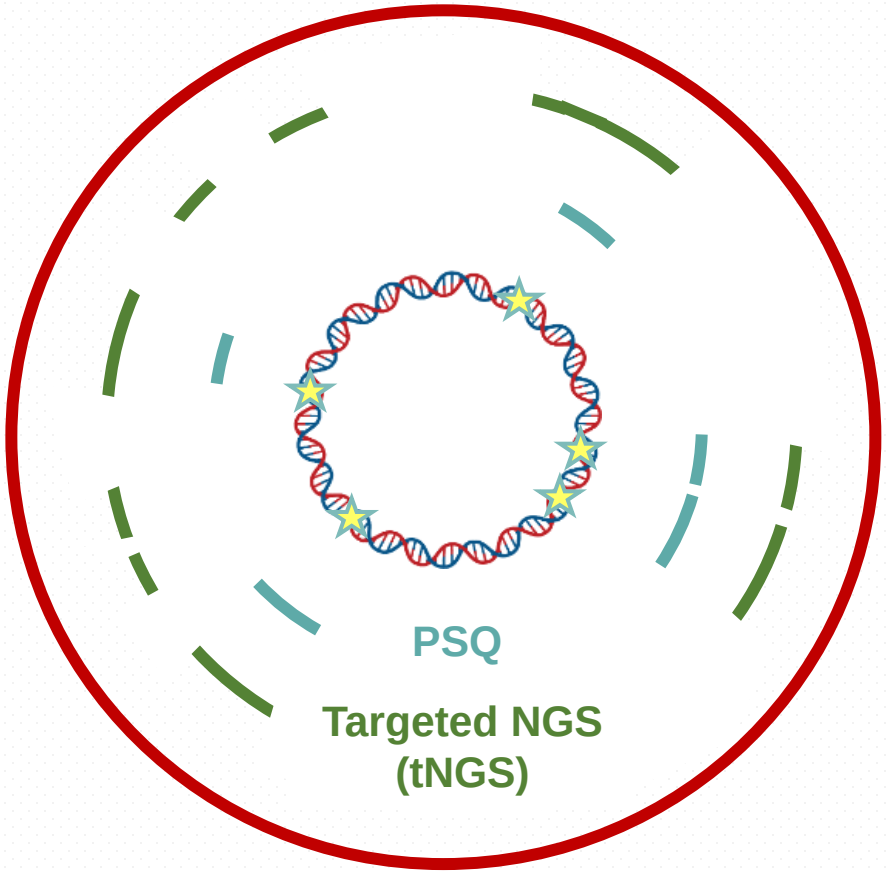
Pyrosequencing (PSQ)

Sanger Sequencing

Targeted Next Generation Sequencing (tNGS)

Whole Genome Sequencing (WGS)

Molecular methods of resistance detection: difference in purview



Whole Genome Sequencing (WGS)

Adapted from Whole Genome Sequencing for TB Drug Resistance at the TB DST Reference Center, 2024, APHL

Pyrosequencing

- Highly sensitive
- Amplifies short sequences of base pairs
- Quick turnaround (3-5 days)
- Can be performed on PCR+ sediment with very low smears
- CAHD uses if WGS unobtainable (sediment or mixed culture)
- Wadsworth tests new patient PCR+ and to confirm primary or fluroquinolone resistance

Sanger Sequencing

- Requires ~10x more DNA than pyrosequencing
- Amplifies longer sequences
- Turnaround (5-7 days)
- Can be performed on PCR+ sediment with low smear count
- CDC sequences *rpoB* if tNGS fails or delayed
- Wadsworth sequences *pncA* (PZA) by this method

tNGS Sequencing

- Requires slightly more DNA than Sanger
- Amplifies longer and more sequences
- Turnaround 7-10 days
- Can be performed on PCR+ sediment with smear positivity of at least 1-10/field
- Can detect subpopulations as low as 10%
- Primary CDC sequencing scheme
- CAHD plans to bring online in near future
- FLHD currently performs

Genetic Loci Sequenced through tNGS MDDR (CDC)

Genetic Locus	Associated Drug
RRDR within the *rpoB* gene with the addition of two codons outside of the RRDR	Rifampin (RMP)
inhA, *katG*, *fabG1*	Isoniazid (INH)
embB	Ethambutol (EMB)
pncA	Pyrazinamide (PZA)
gyrA, gyrB	Fluoroquinolones
rrs	Amikacin Capreomycin Kanamycin
eis	Kanamycin

Additional Genetic Loci Sequenced through tNGS

Genetic Locus	Associated Drug
atpE *rv0678* *pepQ*	Bedaquiline
rv0678 *pepQ*	Clofazimine
rplC *rrl* (partial)	Linezolid

WGS Sequencing

- Requires culture (sediment cannot amplify)
- Sequences practically entire genome
- Turnaround 12-16 days
- All major laboratories perform (CA, NY, FL, TX, CDC)
- Can be used for both DST and TB species ID
- Extremely helpful for epidemiological investigation

TB WGS-DST targets (CAHD)

Red text- new targets in WGS not covered by PSQ

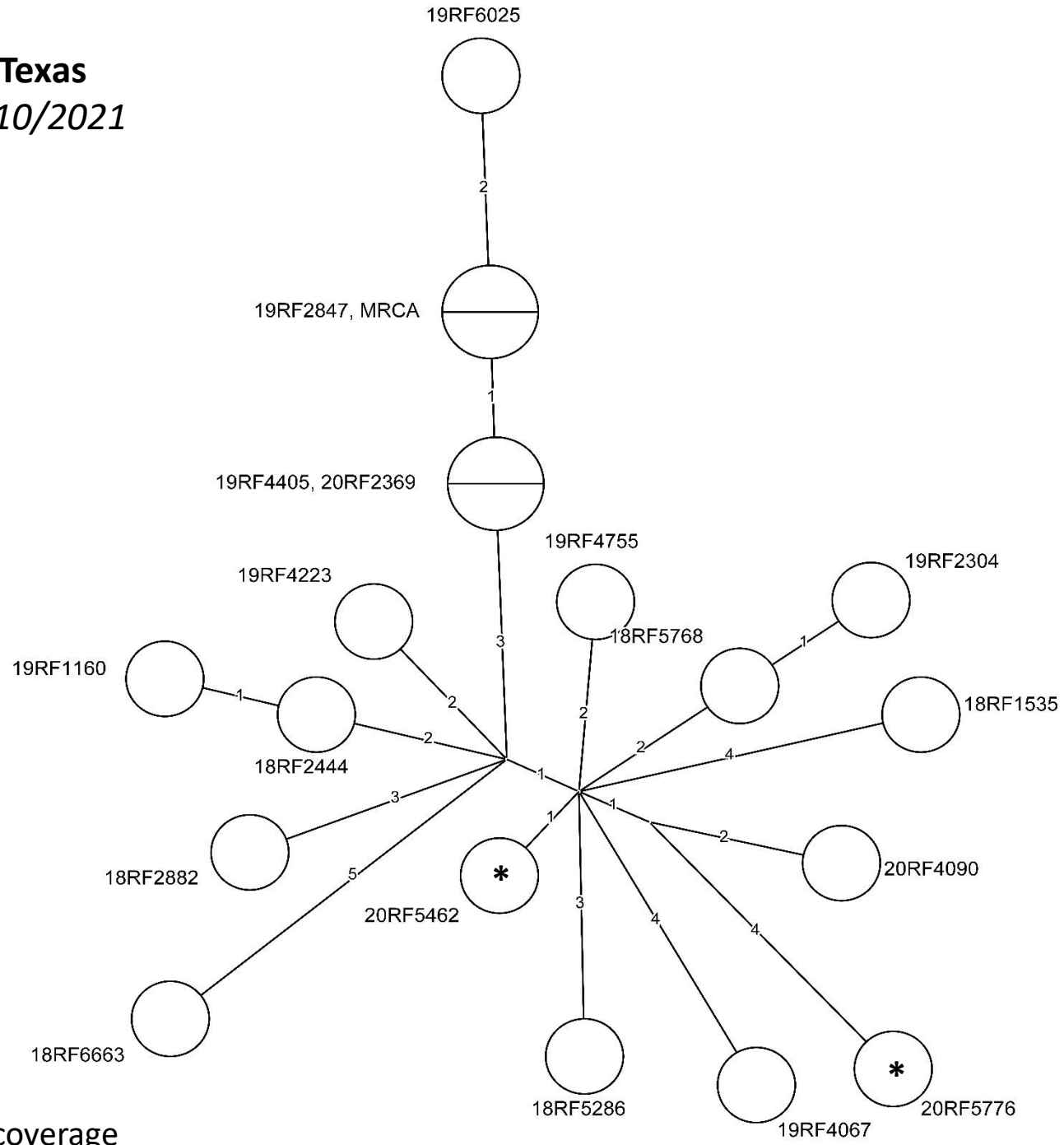
	Drug	PSQ loci	Added WGS loci
1st line	Isoniazid	*katG, fabG1/inhA, ahpC*	*= PSQ* - ahpC*
	Rifampicin	*rpoB*	*= PSQ**
	Pyrazinamide	*pncA* (*M. bovis* ID only)	*= PSQ* + pncA* (PZA R)
	Ethambutol	-	*embA, embB*
2nd line	Amikacin/Kanamycin	*rrs*	*= PSQ* + eis*
	Capreomycin	*rrs*	*= PSQ* + tlyA*
	Fluoroquinolones	*gyrA*	*= PSQ* + gyrB*
	Ethionamide	*fabG1/inhA*	*= PSQ* + ethA*
Other	Bedaquiline	-	*Rv0678 (mmpR), pepQ, atpE, mmpL5, mmpS5*
	Clofazimine	-	*Rv0678 (mmpR), pepQ, atpE, mmpL5, mmpS5*
	Linezolid	-	*rplC, rrl*
MTBC ID confirmation		*Yes*	*Yes*

*For targets included in PSQ, the genomic ranges covered in WGS DST assay are broader and include FL genes & promoter regions (with some exceptions for reporting ranges).

MTBC000025 in Texas

Results received 2/10/2021

Analysis updated with 20RF5776 and 20RF5462 (isolates from the same patient)



Excluded isolates:

19RF3857 – contaminated
18RF6976 – contaminated
18RF6084 – contaminated
18RF4713 – contaminated
18RF3636 – low sequence coverage

**Isolates denoted with an asterisk are from the same patient. Isolate 20RF5462 was collected from a sputum specimen and 20RF5776 was collected from a urine sample.*

Additional DST

- Very few laboratories perform conventional testing on the newer antibiotics
- Molecular targets typically relied upon
- Wadsworth will report clinical breakpoint results for bedaquiline (1.0 µg/mL), clofazimine (1.0 µg/mL), and linezolid (1.0 µg/mL) by MGIT 960
- Can also perform MICs

Thank you!

jan.owen@dshs.texas.gov
benjamin.alpers@dshs.texas.gov