Laboratory Diagnosis of TB

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Objectives

- Describe laboratory methods used for the detection, identification, genotyping, and drug susceptibility testing of *M. tuberculosis*

Outline

- How to collect
- Smear
- Growth-based testing
- Drug Susceptibility testing

When to collect specimens?

- Sputum is the most common specimen
  - Collect 5-10 mls of an early morning specimen, prior to eating
- Usually 3 specimens on 3 different days are recommended for diagnosis
  - At least 8 -24 hours apart

Why first thing in morning?

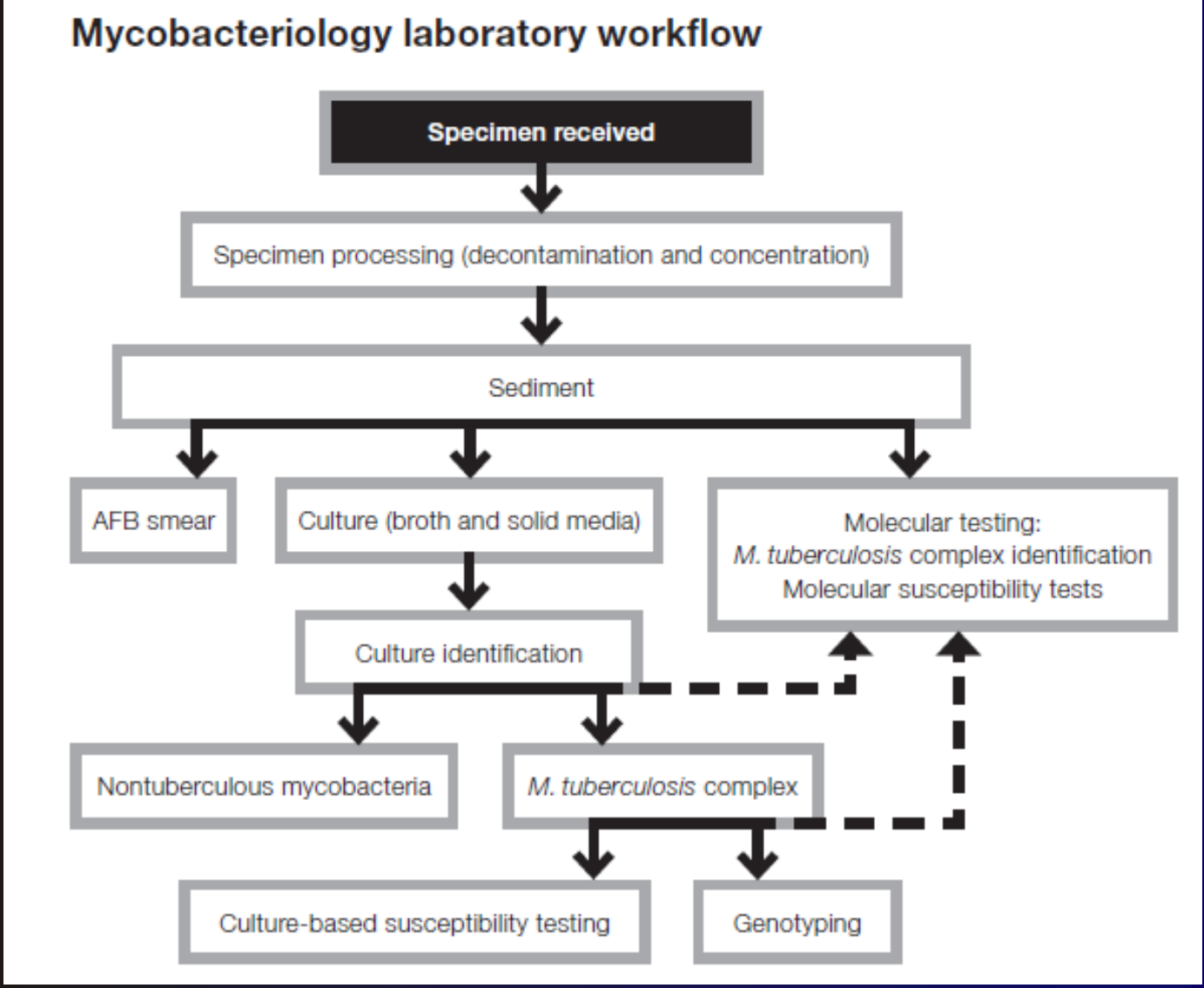
Study	Specimen Type	Total	% Positive
1	Overnight	160	*85,0*
	Spot	160	*51,8*
2	Overnight	181	*31,5*
	Spot	179	*13,9*

1 PANDE et al., Indian J Tuberc 21:1974, 192
2 R.VALLADERS & R.URBANCZIK, Instituto Nacional de TB, Venezuela 1968 – 1969 (not published)

How to collect them?

- https://Sputum Video

What happens after?

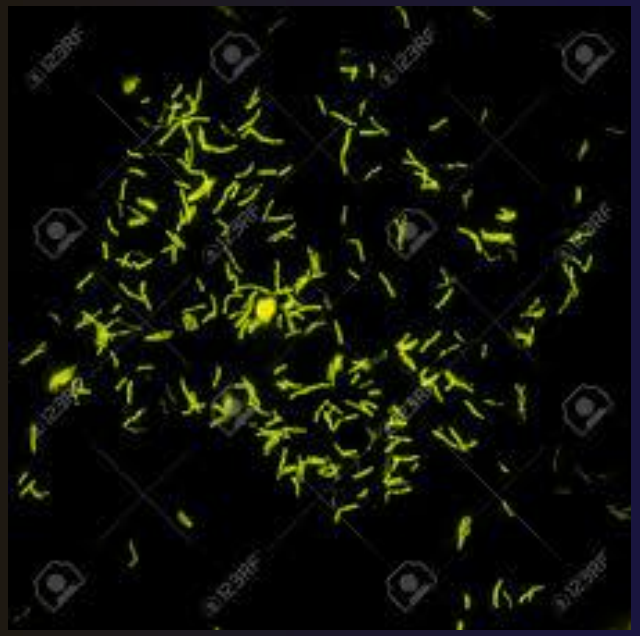


Sputum Staining

- 24 hours after receipt of specimen
- Detection limit 5,000 – 10,000 AFB per mL of sputum
- Fluorochrome staining more sensitive than Ziehl-Neelsen

Sputum Staining

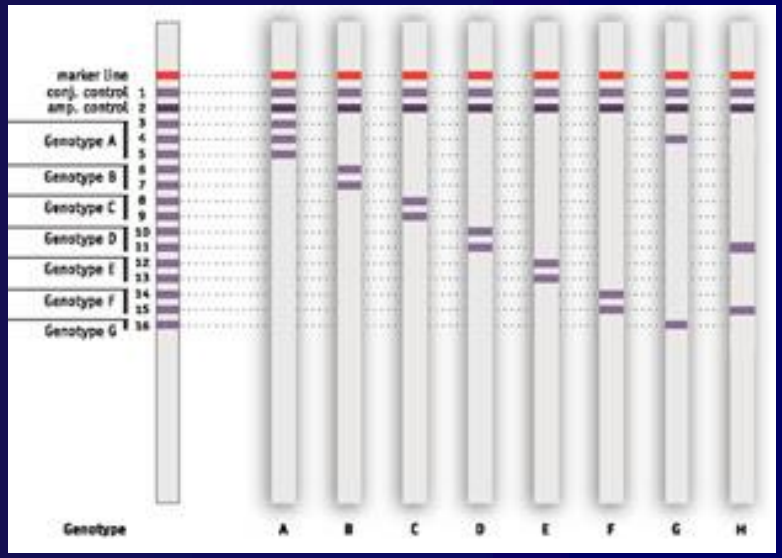
- Fluorochrome stains
  - AFB appear yellow against a black background



- Carbol fuchsin-based stains
  - Two types: Ziehl-Neelsen and Kinyoun. (NOTE: ZN is more sensitive)

NAAT Test


Amplified MTD


marker line
conj. control
amp. control
Genotype A
Genotype B
Genotype C
Genotype D
Genotype E
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GeneXpert

Direct Detection Using Nucleic Acid Amplification (NAA)

- Rapidly identify a specimen via DNA
- Benefits
  - Earlier lab confirmation
  - Earlier respiratory isolation and treatment initiation
  - interruption of transmission
- Perform at least 1 NAA per patient
- A single negative NAA does not exclude TB

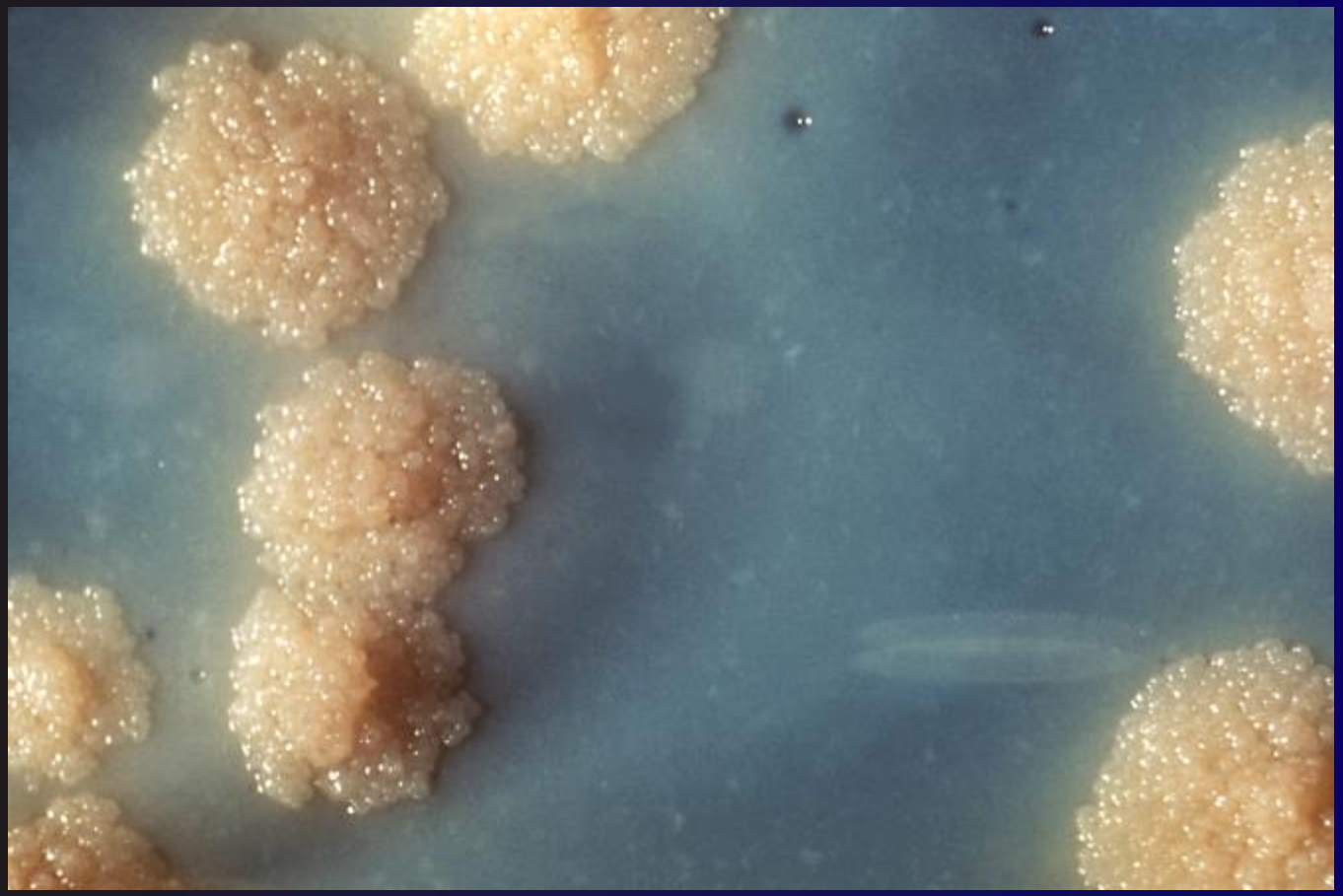
Culture

- Remains reference standard for confirming diagnosis of TB
- Culture all specimens, even if smear or NAA negative
- Culture monthly until conversion, i.e., 2 consecutive negative cultures

Culture: Solid Media (Agar)

- Middlebrook 7H10 or 7H11 which are agar- based
- Advantage that organisms (colonies) can be seen
- If there is mixed growth or contamination, picking individual colonies can allow you to obtain a pure culture.

7H-10 Media



Culture: Liquid Media

- MGIT 960
  - Results in about 1 week
  - Based on the fluorescence produced from reduced oxygen due to microbial growth
  - Fluorescence then converted to “growth units” (GU).
    - More GU indicates more growth.
    - GU = 400 within, the DST is valid
    - GU<100, the organism is interpreted as being susceptible;
    - GU is ≥100, the organism is considered resistant.

MGIT Incubator

Culture: Liquid Media

- VersaTREK: measures change in oxygen due to growth
- Sensititre: 96-well microbroth dilution plate using colonies grown on solid media
- MODS (microscopic observation drug susceptibility): based on visualization of the cording morphology

Culture: Liquid Media

- MGIT 960, VersaTREK, Sensititre
- Results in about 1 week
- Based on the fluorescence produced from reduced oxygen in the MGIT medium due to microbial growth
- The fluorescence generated is then converted to “growth units” (GU). In general, more GU indicates more growth.
- • When the growth control generates GU to 400 within 4-14 days, the DST is valid

Drug-Susceptibility Testing

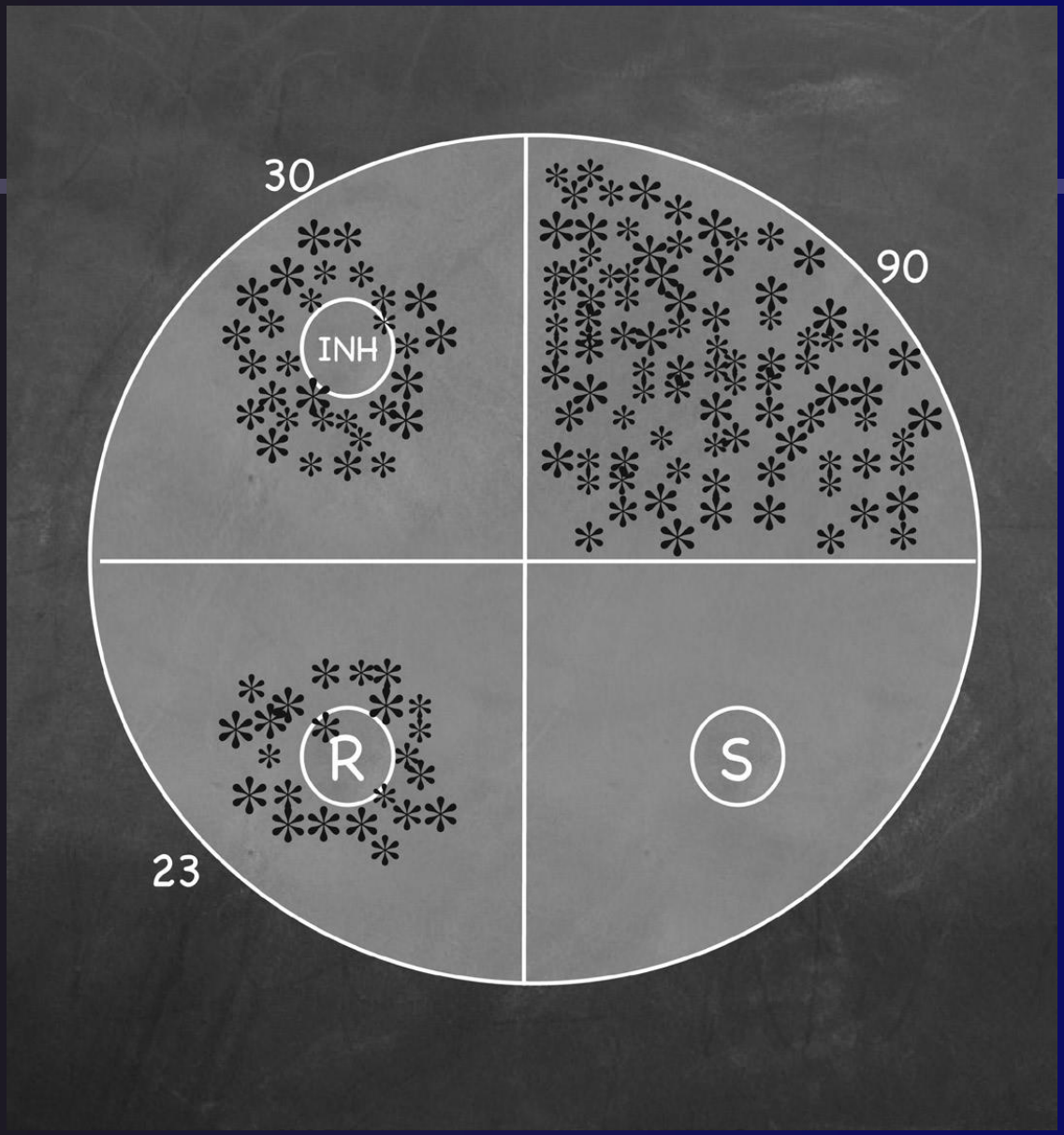
- Conduct drug-susceptibility testing on initial *M. tb* isolate

- Repeat for patients who
  - Do not respond to therapy or
  - Have positive cultures despite 3 months of therapy
  - Retreatment

Methods for DST

- Agar proportion compares growth on agar media with and without one of the four primary drugs

- Broth based (MGIT, Trek)
  - Requires inoculation of the strain in broth with each of the (5) primary drugs, plus control vial
  - Growth of the strain in a vial with a drug indicates resistance to that drug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Drug-Susceptibility Testing

Second-line Drug-Susceptibility Testing

Limit to persons at increased risk for drug resistance:

- Have history of treatment with TB drugs
- Had contact with a person with drug-resistant TB
- Demonstrated resistance to first-line drugs
- Has positive smears or cultures despite 3 months of TB treatment

Genotyping

- Spoligotyping (spacer oligonucleotide typing)
- MIRU/VNTR (mycobacterial interspersed repetitive units/ variable number of tandem repeats)
- RFLP fingerprinting (restriction fragment length polymorphism)
- Whole genome sequencing (WGS)

Spoliogotyping

- Gives a result as a number, so to tell if 2 strains are different, just see if they have different numbers
- Not too powerful at discriminating different strains.
- Performed at CDC, using DNA sequencer

MIRU

- Like spoligotyping, the result is a number (24 digits)
- Like spoligotyping, MIRU sometimes doesn’t discriminate between unrelated strains
- 24 locus MIRU protocol is in use, making it more powerfully discriminatory

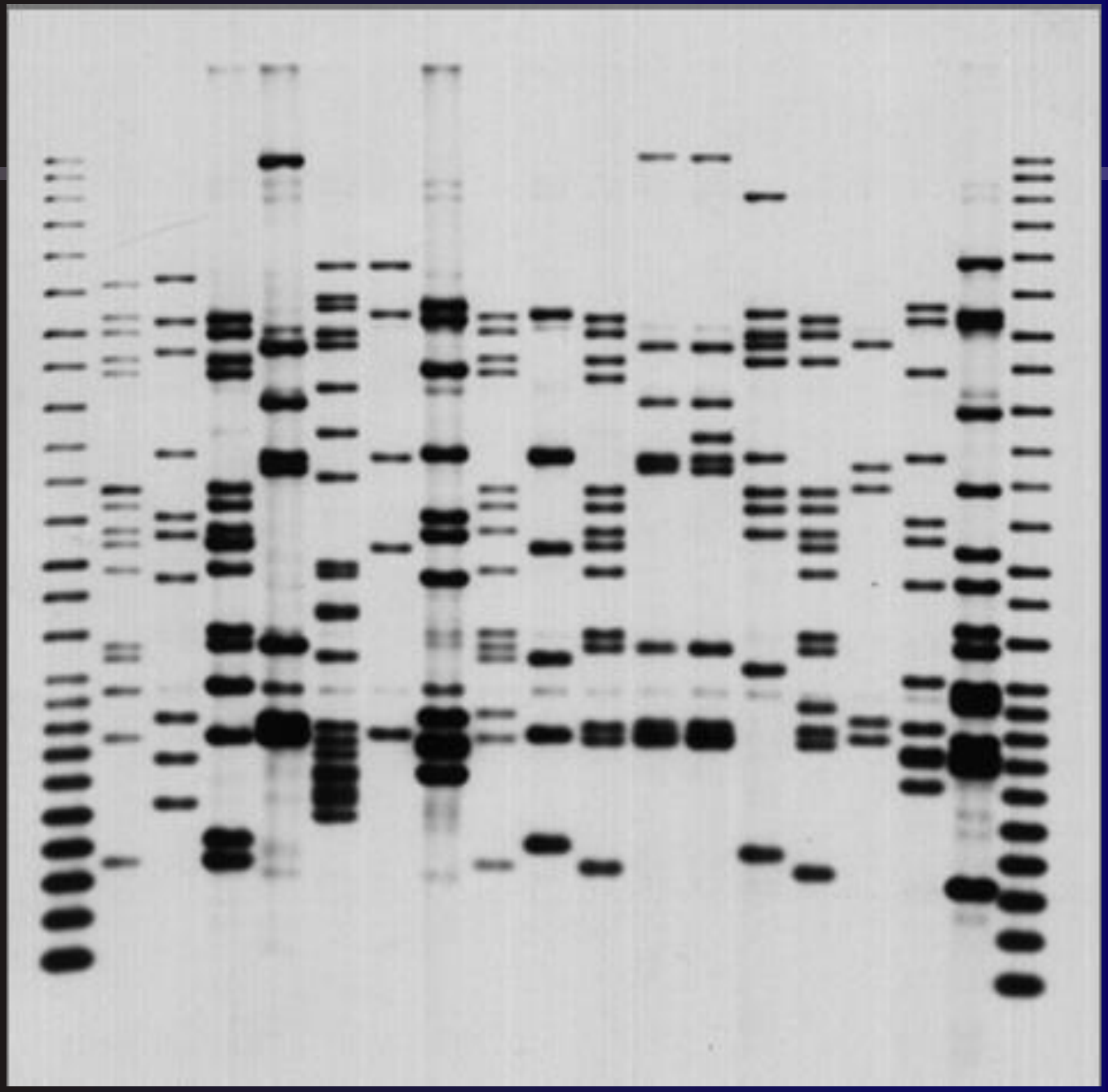
MIRU and spoligotyping together

- Both PCR-based, so small amount of DNA needed

- If 2 strains of M. tuberculosis are different, unlikely that they will have the same spoligotype AND MIRU type
  - Possible exceptions include Manila strains and Chinese “Beijing” strains

RFLP

- DNA electrophoresis, and requires a lot of DNA
- Complicated procedure that takes ~ a week
- Result is a visual pattern making database input challenging
- Most powerful methods
- Performed only on strains that match by MIRU and spoligo, and only by request



Whole Genome Sequencing

- Other methods interrogate <1% of genome
- WGS looks at entire genome
- Can be used for improved molecular-guided TB surveillance and control to better characterize recent transmission

TABLE 1.

Mycobacteriology laboratory services

Test	Expected turnaround time (from specimen receipt at laboratory)	Comments
AFB smear	1 day	Fluorochrome staining is more sensitive than carbol-fuchsin acid fast staining (Ziehl-Neelsen or Kinyoun methods).
Nucleic acid amplification testing (NAAT) For identification of *M. tb* complex	1-2 days	Commercial, FDA-cleared tests and laboratory developed tests available. Excellent sensitivity and specificity for testing smear-positive sediments. Testing smear-negative sediments usually has reduced sensitivity and specificity.
Molecular detection of drug resistance (may also include identification of *M. tb* complex)	1-3 days	Becoming more widely available, particularly for rifampin testing. See Table 5 for more information. New technologies are emerging.
Mycobacterial culture and identification	Positive cultures: average of 2-3 weeks incubation. Smear-negative specimens may take >4 weeks to turn positive. 6–8 weeks to report negative.	When a culture takes 5-6 weeks to turn positive, consider investigation for possible cross-contamination.
Identification of positive cultures	1 day to 1 week for identification of *M. tb* complex, MAC, *M. kansasii*, and *M. gordonae* by DNA probes. Identification of other non-TB mycobacteria may take days or months depending on method used.	Laboratories may batch tests; testing time by DNA probes or MALDI-TOF is less than 2 hours.
Growth-based DST	Liquid broth systems: 1-2 weeks after setting up DST. (4 weeks or longer from specimen receipt at laboratory.) Solid media (agar proportion method): 3-4 weeks.	DST cannot be performed on mixed or contaminated cultures. Laboratories usually perform DST in batches.
Genotyping	MIRU: 2 weeks Spoligotype: 1 month	MIRU is performed at the Michigan TB laboratory. Spoligotyping is performed at CDC. Expedited genotyping may be requested for investigation of outbreaks or cross-contamination.
Interferon gamma release assays (IGRA)	1-2 days (longer if batched)	Usually performed by clinical laboratory (not mycobacteriology laboratory).

Summary

- Don’t forget to get these labs tests for your patients
  - Smear
  - NAAT/Xpert
  - Culture
- Genotyping will be sent by your regional lab