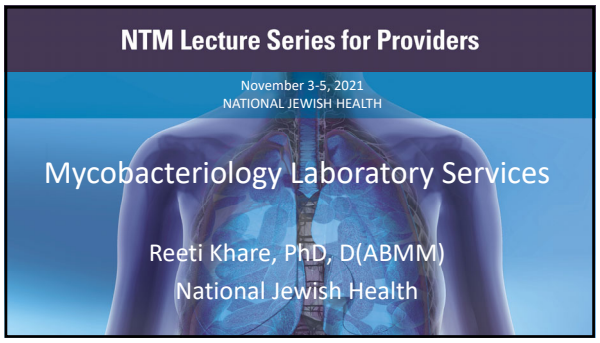

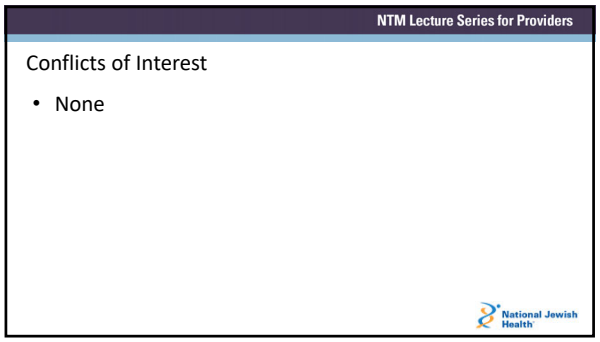

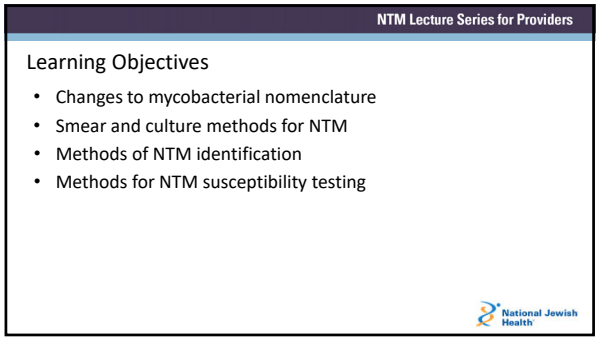

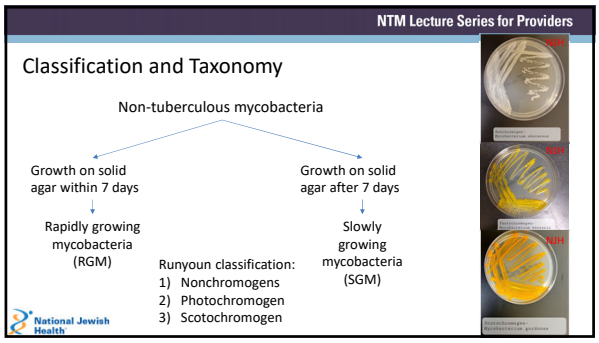

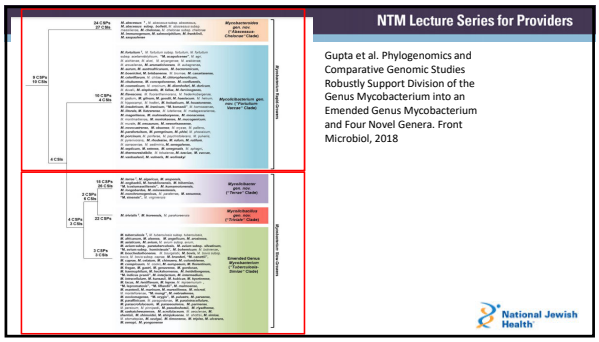

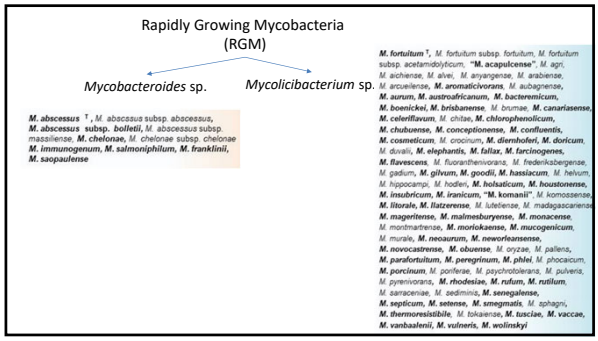

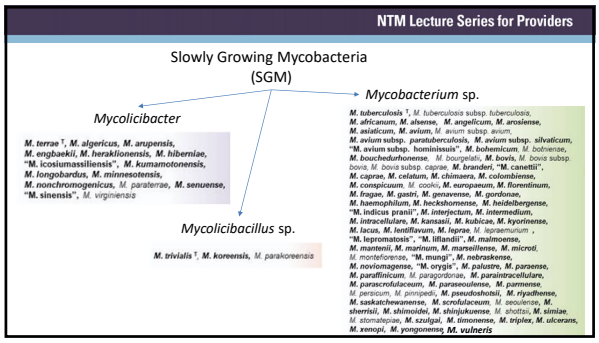

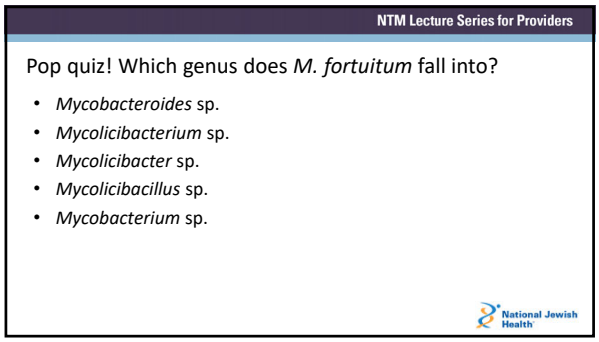

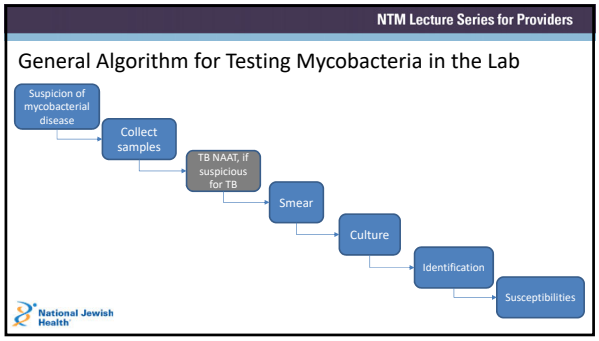

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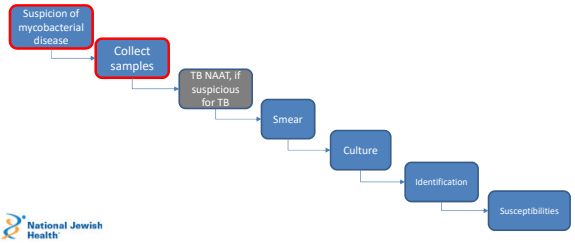
General Algorithm for Testing Mycobacteria in the Lab



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Lab Tests to Detect Mycobacteria

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- NTM exist naturally in the environment, especially water
- NTM can be part of the normal flora[1]
  - Oral flora (likely transient) in 26-33% of people[2]
  - Presence of NTM without progressive disease
- Detection ≠ infection

[1] Thornton et al. The respiratory microbiome and nontuberculous mycobacteria: an emerging concern in human health. European Respiratory Review 2021 30: 200299
[2] Wali et al: The presence of atypical mycobacteria in the mouthwashes of normal subjects: role of tap water and oral hygiene. Ann Thorac Med 2008; 3: pp. 5-8

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Specimen Collection

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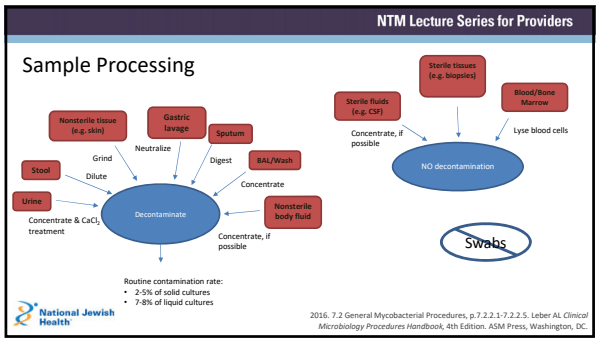
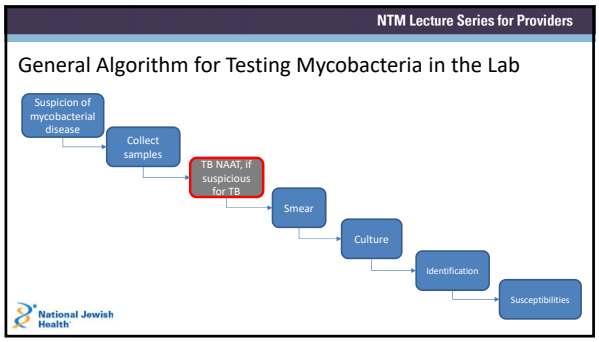
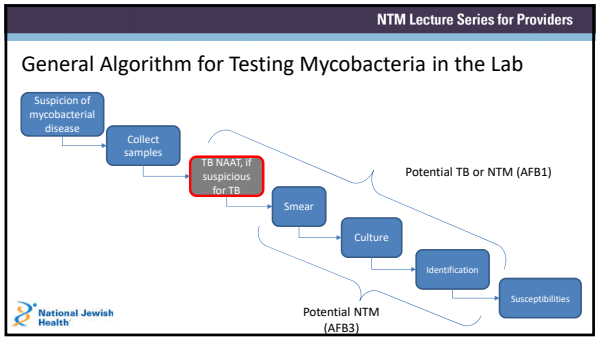
- Multiple sputum specimens
  - Expectorated or induced
  - Recommended: 5-10 ml[1]
  - Positivity over time[2]
  - >= 2 sputum cultures positive is more likely to be clinically significant (as high as 98% agreement for clinically significant MAC)[3]
- BALs/Bronchial washings may be more sensitive than sputum

[1]Warren et al. A minimum 5.0 ml of sputum improves the sensitivity of acid-fast smear for Mycobacterium tuberculosis, Am J Respir Crit Care Med, 2000
[2]van Ingen. Microbiological Diagnosis of Nontuberculous Mycobacterial Pulmonary Disease. Clinics in Chest Medicine, 2015-03-01, Volume 36, Issue 1, Pages 43-54
[3]Daley et al. Treatment of Nontuberculous Mycobacterial Pulmonary Disease: An Official ATS/ERS/ESCMID/IDSA Clinical Practice Guideline. Clinical Infectious Diseases, 2020







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TB-NAAT Assays from Specimen

- NAAT: nucleic acid amplification test
- Lab developed tests
- Cepheid Xpert MTB/Rif
  - Real-time PCR
  - Report and rifampin resistance

https://www.cepheid.com/en_US/tests/Critical-Infectious-Diseases/Xpert-MTB-RIF

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		Xpert MTB/RIF	
Source	Population	Sensitivity (%)	Specificity (%)
Sputum/ Pulmonary	Adult	85 (81 for HIV+)	98
	Children	65 (72 for HIV+)	99
Gastric aspirate	Children	73	98-99
Pleural fluid	Adults	50	99
Peritoneal fluid	Adults	59	97
Cerebrospinal fluid	Adults	70	97
Synovial fluid	Adults	97	94
Lymph node aspirate	Adults	89	86
Lymph node biopsy	Adults	82	79
Urine	Adults	85	97

		Xpert MTB/RIF	
Source	Population	Sensitivity (%)	Specificity (%)
Pulmonary	Adults	96	98
Extrapulmonary	Adults	96	99

[1]Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection. World Health Organization, Geneva, 2020
[2]Rowlinson, Musser, Khare. *Mycobacterium tuberculosis* Complex, Manual of Clinical Microbiology, 13th ed., in review, 2022

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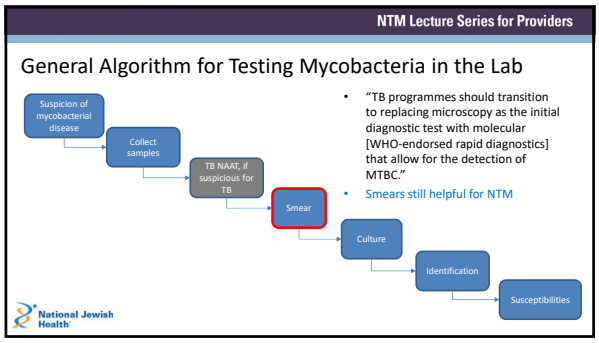

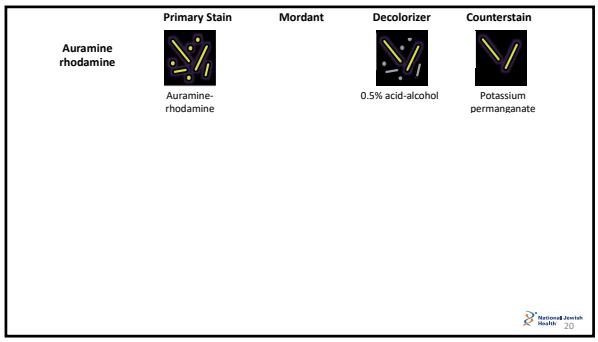

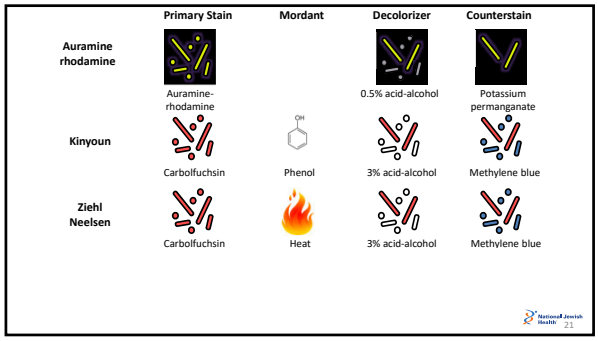

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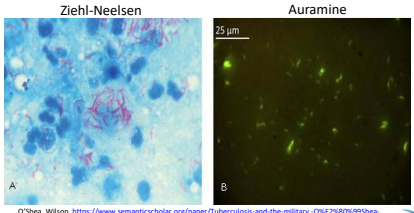
AFB Smears

Ziehl-Neelsen | Auramine



O'Shea, Wilson. https://www.semanticscholar.org/paper/Tuberculosis-and-the-military.-O%E2%80%99Shea-Wilson/6a1850cd7ce1cbde4df1409085862d8a7cd376

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AFB Smears

- Ziehl Neelsen: sensitivity = 20-70%; need ~10^4-10^5 bacilli/ml
- Auramine-rhodamine smears: ~5-10% more sensitive
- Turnaround time: 24 hours

Somoskovi et al. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2925666/#R2
Cattamanchi et al. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2754584/
Singh, Parija. https://www.ncbi.nlm.nih.gov/pubmed/10772577
Azadi et al. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5897959/
Ghiasi et al. https://link.springer.com/article/10.1007/s40475-015-0043-1

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	Primary Stain	Mordant	Decolorizer	Counterstain
Auramine rhodamine	Auramine-rhodamine		0.5% acid-alcohol	Potassium permanganate
Kinyoun	Carbolfuchsin	Phenol	3% acid-alcohol	Methylene blue
Ziehl Neelsen	Carbolfuchsin	Heat	3% acid-alcohol	Methylene blue
Gram stain	Crystal violet	Iodine	Alcohol	Safranin

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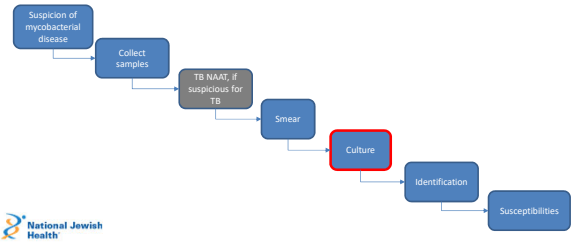
NTM Lecture Series for Providers

General Algorithm for Testing Mycobacteria in the Lab



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Culture Techniques

- Liquid cultures
  - Supplements
    - Sugars (dextrose or glucose)
    - Oleic acid
    - Catalase
  - Antibiotic cocktails
  - 10-15% more sensitive than solid cultures.
  - More rapid growth compared to solid culture

VersaTREK Myco

https://www.fishersci.com/shop/products/versatrek-myco-media-6/Y711142

MGIT tubes

Positive MGIT

Positive MGIT under UV

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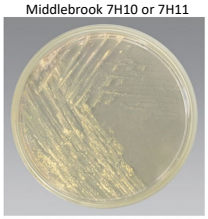
Culture Techniques

- Solid culture
  - Lowenstein Jensen agar
    - Contains egg and malachite green
  - Middlebrook agar
    - Contains casein hydrolysate (for MDR TB)

LJ

https://www.fishersci.ca/shop/products/lowenstein-jensen-medium-lj/p-4523753

Middlebrook 7H10 or 7H11



https://www.fishersci.ca/shop/products/remel-middlebrook-7h11-agar/r01605

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Culture Conditions

- Culture incubated for 6-8 weeks
- Temperatures
  - 35-37ºC: routine
  - 42ºC: *M. xenopi*
  - 30-32ºC: *M. marinum, M. haemophilum, M. ulcerans*
- Supplements
  - Hemin, ferric ammonium citrate – *M. haemophilum*
  - Egg yolk – *M. ulcerans*
  - Mycobactin J – *M. genavense, M. avium* subsp. *paratuberculosis*

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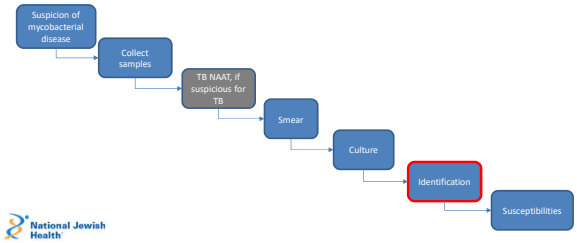
General Algorithm for Testing Mycobacteria in the Lab



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Identification Techniques

- Nucleic acid hybridization
- PCR-based line probes
- MALDI-TOF mass spectrometry
- Sequencing

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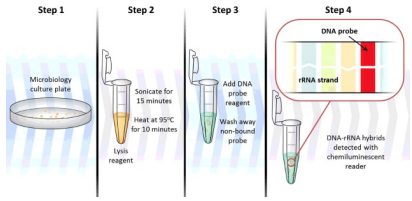
Nucleic Acid Hybridization (i.e. “probes”)



Caulfield, Wengenack. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, Volume 4, August 2016, Pages 33-43

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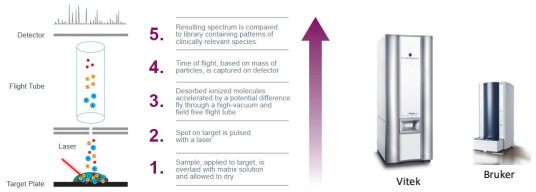
MALDI-TOF Mass Spectrometry



https://www.beckmancoulter.com/products/microbiology/maldi-tof-mass-spectrometry

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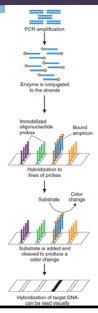
Line Probe Assays (LPA)

- Step 1: PCR
- Step 2: Amplicons are bound onto a membrane containing capture probes for identification and drug resistance genes
- Step 3: Pattern of binding is read



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Khare. Guide to clinical and Diagnostic Virology, 2019

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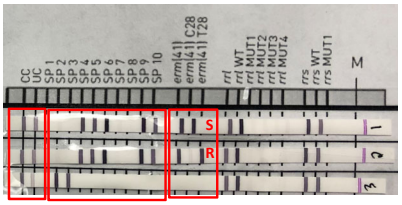
LPA Results



M. abscessus subsp. *abscessus*

M. abscessus subsp. *abscessus*

M. chimaera

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Sanger Sequencing

- Step 1: amplify a target gene
  - Most common gene for identification
    - *rpoB* – encodes subunit of bacterial RNA polymerase[1]
    - 16S –rDNA encoding the 30S ribosomal rRNA
    - 16S-23S ITS – intergenic spacer region
    - *hsp65* – encodes a bacterial heat shock protein
- Step 2: sequence the amplified gene
- Step 3: compare the sequence to a database

Simmon et al. Simultaneous Sequence Analysis of the 16S rRNA and *rpoB* Genes by Use of RipSeq Software To Identify *Mycobacterium* Species. JCM, 2010

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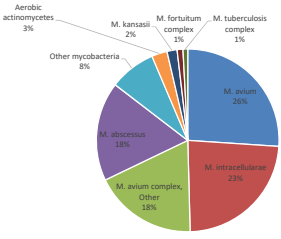
Most Common Organisms Seen in Our Lab



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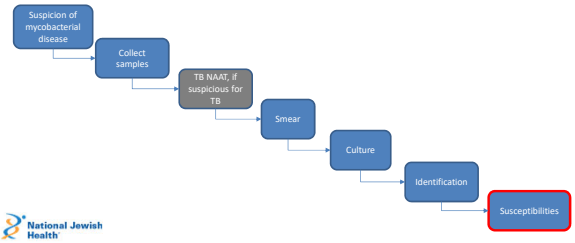
General Algorithm for Testing Mycobacteria in the Lab



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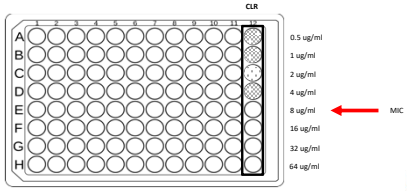
Antibiotic Susceptibility Testing (AST)

- Broth microdilution testing



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AST Interpretation

- Minimum Inhibitory concentration = MIC value
- Interpretation
  - S, I, R = Susceptible, Intermediate, Resistant
  - Defined by CLSI M62, 2018

MAC

Antimicrobial Agents	MIC, µg/mL		
	S	I	R
First Line			
Clarithromycin	≤ 8	16	≥ 32
Amikacin (IV)	≤ 16	32	≥ 64
Amikacin (liposomal, inhaled)	≤ 64	–	≥ 128
Second Line			
Moxifloxacin	≤ 1	2	≥ 4
Linezolid	≤ 8	16	≥ 32

Rapid growingly mycobacteria

Antimicrobial Agent	MIC, µg/mL		
	S	I	R
Amikacin (IV)	≤ 16	32	≥ 64
Cefoxitin	≤ 16	32–64	≥ 128
Ciprofloxacin	≤ 1	2	≥ 4
Clarithromycin	≤ 2	4	≥ 8
Doxycycline	≤ 1	2–4	≥ 8

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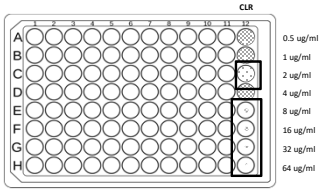
Antibiotic Susceptibility Testing (AST)

- Broth microdilution testing



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Newer Drugs

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- Omadacycline[1]
  - Expect low MIC values: 0.06-1 ug/ml (at 100% inhibition)
  - Isolates resistant to tigecycline can still be susceptible to omadacycline
- Tedizolid MICs 1-8 dilutions lower than linezolid[2]
- Bedaquiline: expect low MICs (<0.016 – 0.125 ug/ml)[3]
- Delaminid: Low for *M. kansasii*, but high for *M. abscessus* isolates that were tested[3]

[1]Brown-Elliott and Wallace. *In Vitro* Susceptibility Testing of Omadacycline against Nontuberculous Mycobacteria. AAC, 2021
[2]Brown-Elliot and Wallace. In Vitro Susceptibility Testing of Tedizolid against Nontuberculous Mycobacteria. JCM, 2017
[3]Kim et al. In Vitro Activity of Bedaquiline and Delamanid against Nontuberculous Mycobacteria, Including Macrolide-Resistant Clinical Isolates. Antimicrob Agents Chemother. 2019

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Turnaround Time

- Slow Growers:
  - Read at 7-14 days
  - Fastidious species at 3-4 weeks
- Rapid Growers:
  - Most drugs read at 3-5 days
  - Clarithromycin: read at 14 days
- Other Factors
  - Insufficient growth? Needs subculture
  - Mixed culture? Needs re-isolation 😱

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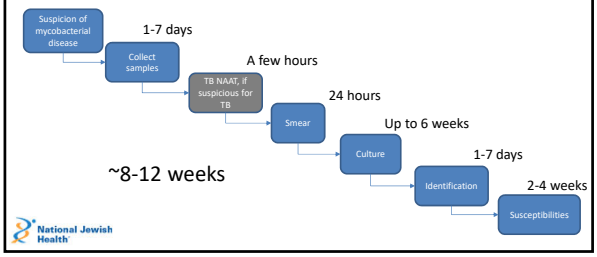
General Algorithm for Testing Mycobacteria in the Lab



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Antibiotic Susceptibility Testing (AST)

- Gene-based markers of drug resistance
  - Mutations in *rrs*: aminoglycosides
  - Mutations in *rrl*: constitutive macrolide resistance
  - Presence of functional *erm*(41): inducible macrolide resistance.
- Faster assessment of inducible resistance
  - Molecular testing TAT is ~1 day; inducible resistance testing ~>14 days.
  - 100% correlation between molecular analysis and inducible macrolide susceptibility results[1-3]

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[1]Hanson et al. Rapid Molecular Detection of Inducible Macrolide Resistance in Mycobacterium chelonae and M. abscessus Strains: a Replacement for 14-Day Susceptibility Testing? JCM 2014
[2]Bastian et al. Assessment of Clarithromycin Susceptibility in Strains Belonging to the Mycobacterium abscessus Group by erm(41) and rrl Sequencing. Antimicrob Agents Chemother. 2011
[3]Sharma et al. A real-time PCR assay for rapid identification of inducible and acquired clarithromycin resistance in Mycobacterium abscessus. BMC Infect Dis. 2020

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Learning Objectives

- Changes to mycobacterial nomenclature
  - New names for 5 new genera; is not fully adopted
- Smear and culture methods for NTM
  - Depends on good samples
  - Sample processing, smears, liquid culture, solid culture
- Methods of NTM identification
  - Nucleic acid hybridization
  - PCR-based line probes
  - MALDI-TOF-MS
  - Sequencing
- Methods for NTM susceptibility testing

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