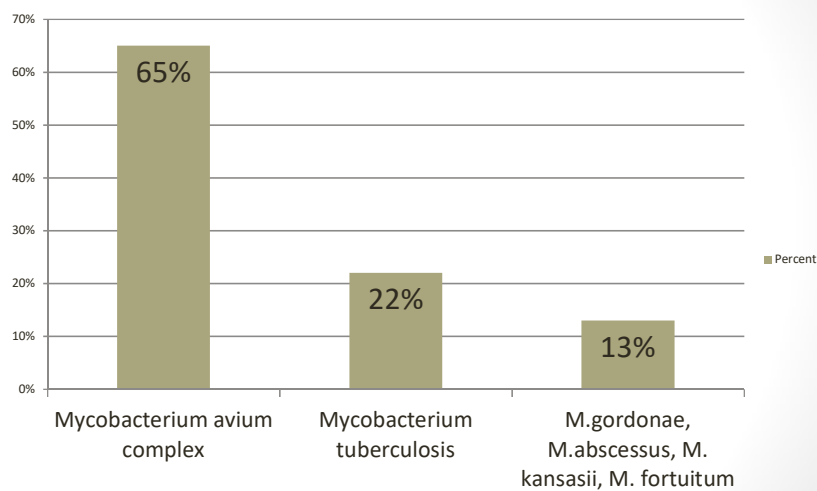


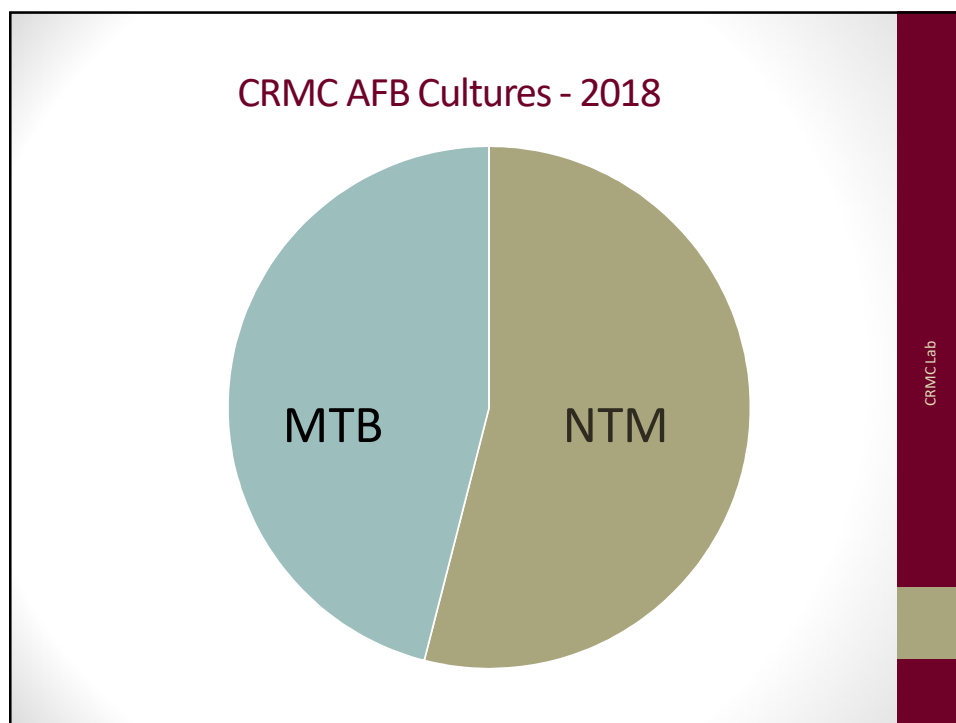
Nontuberculous Mycobacteria (NTM) Laboratory Identification Methods

Lung Symposium
October 26, 2019

Speaker: Marilyn Mitchell, MT(ASCP)MS
Supervisor: CRMC Microbiology Lab

Mycobacteria species distribution in patients – CRMC Microbiology Dept. - 2018





- ### AFB Culture Components
- **AFB smear**
 - Fluorescent stain, then Ziehl-Neelson stain for confirmation of new positives or if specimen is tissue
 - **Liquid Media culture**
 - MGIT (Mycobacteria Growth Indicator Tube) broth
 - **Solid Media culture**
 - LJ slant (Lowenstein Jensen egg media)
 - If positive for a mycobacteria – may need to send out for susceptibility testing

Method Sensitivities – CDC data

Smear: 5,000-10,000 bacilli/ml to be detected

Culture: 10-100 bacilli/ml to be detected

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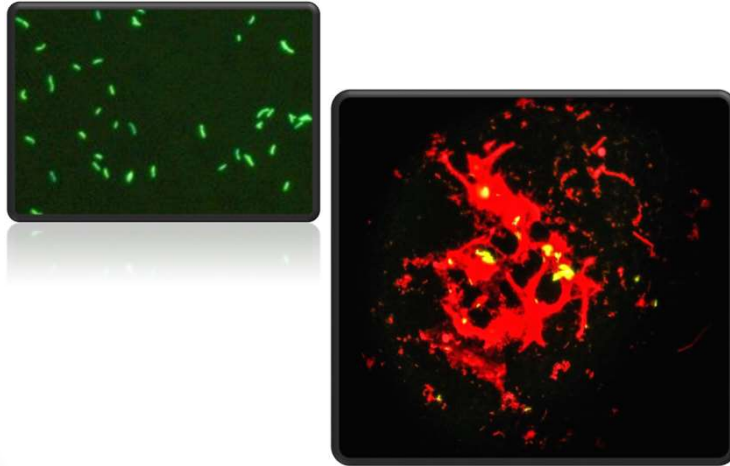
AFB Characterization by Morphology

Where does the name come from?

- Mycobacteria are **rod-shaped** bacteria
- **Acid-fast** – unusual lipids in their cell walls
 - Waxy cell wall (**mycolic acid**)
 - Carbol-fuchsin solution stains the bacteria red
 - Resist decolorization
- Fluorescent microscopy staining may also be used to identify *Mycobacterium*.

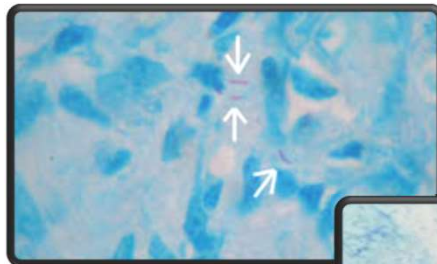
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AFB Auramine O Fluorescent Stain



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AFB Ziehl-Neelsen stain



Must look at 300 microscope fields to call negative



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AFB smear Quantitation

1+	=	3-30 Organisms/Slide (1-9 per 100 fields, but scan 300 fields)
2+	=	1-9 Organisms/ 10 fields
3+	=	1-9 Organisms/field
4+	=	>9 Organisms/field
+/-	=	Only reported in our computer workup notes – Recheck another smear. 1-2 Organisms/slide (eg. – 1-2 per 300 fields)

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Growth of AFB in Liquid Media - MGIT

Depends on species

- Categorized by speed of growth
 - **Rapid growers - growth in less than 7 days**
 - M. fortuitum, M. chelonae, M. abscessus
 - **Slow growers – growth in more than 7 days:**
 - MTB and M.avium
- Affected by :
 - Initial inoculum size
 - Enrichment of media
 - Temperature

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AFB Growth – Liquid and Solid Media

MGIT: Mycobacteria
Growth Indicator Tube –
6 weeks



MGIT: Fluoresce
when Positive
Growth



Growth on Solid
Media – LJ Slant –
8 weeks



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And if Culture becomes positive?

Liquid or solid media becomes positive

- Confirm it is AFB – AFB stained slide
- Next day perform Genprobe Accuprobe culture ID
 - **85% are MAC or MTB**
 - If positive, subculture and send out for susceptibilities if needed.

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If Negative for MTB and MAC -

Vitek MS Maldi-TOF

- Colonies on solid LJ media work best
- AFB in liquid media is a little more difficult

Send to ARUP reference lab

- Difficult identifications
- When sensitivities are needed

NATIONAL JEWISH HEALTH in Denver

They help with difficult IDs and sensitivities
Next generation sequencing



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Method Improvements for Cultures over 25 years

College of American Pathologists – our governing agency, requires us to use rapid methods when available

- AFB Smear
 - Old AFB smear - Ziehl-Neelson
 - Newer - Fluorescent stain and fluorescent microscope
- AFB Culture
 - Older - Solid media (LJ slant)
 - Newer - Liquid MGIT tube plus solid
- AFB Identification
 - Older - Biochemical identification that takes weeks
 - Newer - DNA probes and Maldi-TOF

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Direct Specimen Testing –Before Culture is Grown Game Changer

Cepheid Direct MTB PCR

- AFB smear positive specimens – 98% sensitive
- AFB smear negative specimens – 72% sensitive
- FDA approved for respiratory specimens.
 - Pleural fluids reported with a disclaimer.
- Cepheid method - only identifies *Mycobacteria tuberculosis*

Future Direct PCR test will include

- Mycobacterium avium complex
- Mycobacterium tuberculosis
- Pan-Mycobacteria species

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Futures: ID Direct from Specimen

Direct Mycobacteria method similar to the Direct Cocci PCR assay.

- 3 reportable results:
 - Pan-Mycobacteria – any Mycobacteria species
 - M.tuberculosis complex
 - M.avium complex

Saves about 2 weeks in identification of MTB AND MAC

- Same instrument as the test for Cocci PCR – use the BD Max

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Test Orders Summary

- You order AFB Culture (CXAFB):
 - AFB smear and AFB Culture
 - If positive: Worked up is performed using best practices
 - Several tools for accurate, rapid testing
- Direct MTB PCR – requires a separate order
 - Not performed as part of AFB culture
 - Infection Preventionists contact the lab when this is needed
 - Direct PCR currently available for MTB
 - Direct PCR available in a few months for MAC

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Direct Specimen Testing -- CDC suggestions

NAA testing should be performed on a least one respiratory specimen

- On each patient with signs and symptoms of pulmonary TB
 - If a diagnosis of TB is being considered but has not yet been established
 - If test result would alter case management or TB control activities, such as contact investigation.

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Direct Specimen Testing -- CDC suggestions

CDC also suggests

- Don't base diagnosis on a single Negative NAA test result
- Use in context with clinical situation
- A negative NAA
 - may help direct consideration to an alternative diagnosis
 - possibly preventing unneeded TB disease treatment

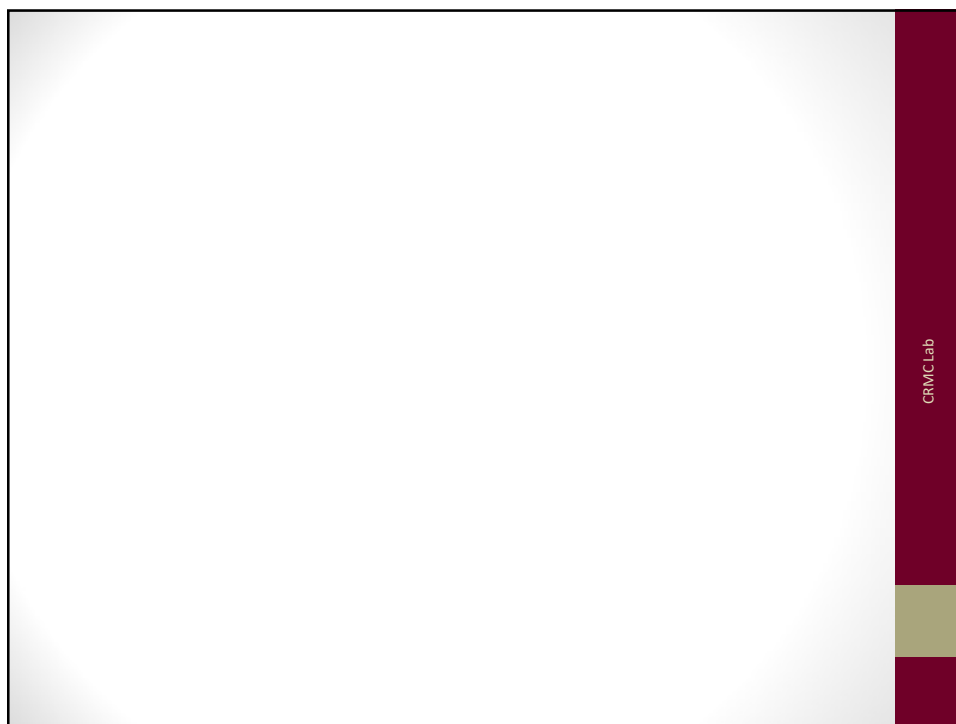
CULTURE REMAINS THE GOLD STANDARD for lab confirmation of TB disease – plus we need the growing bacteria to perform susceptibility testing.

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Method Improvement Goals

- Improved patient outcomes
- Earlier treatment initiation
- More effective public health intervention – investigation for contacts, etc.
- Needs AFB Culture also

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AFB Culture Specimen

- **Respiratory Specimens:**
 - 5 ml minimum – Sputum, Induced sputum, BAL – need to digest, decontaminate and concentrate
 - Clean up with NALC/NaOH
 - Can collect 1 specimen every 8 hours for 24 hours
- **Body fluids** - pleural fluids, CSF
- **Tissues** – lung nodules
- **Bone, bone marrow**
- **Skin biopsy**

Please do not send swabs – if necessary to use swabs, send at least 2 very saturated swabs for the AFB culture