An Ode to the "Cord:" Mycobacteriology Diagnostic Testing Review

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NACMID conference

September 25, 2023

Disclosures

- NACMID speaker's Honorarium

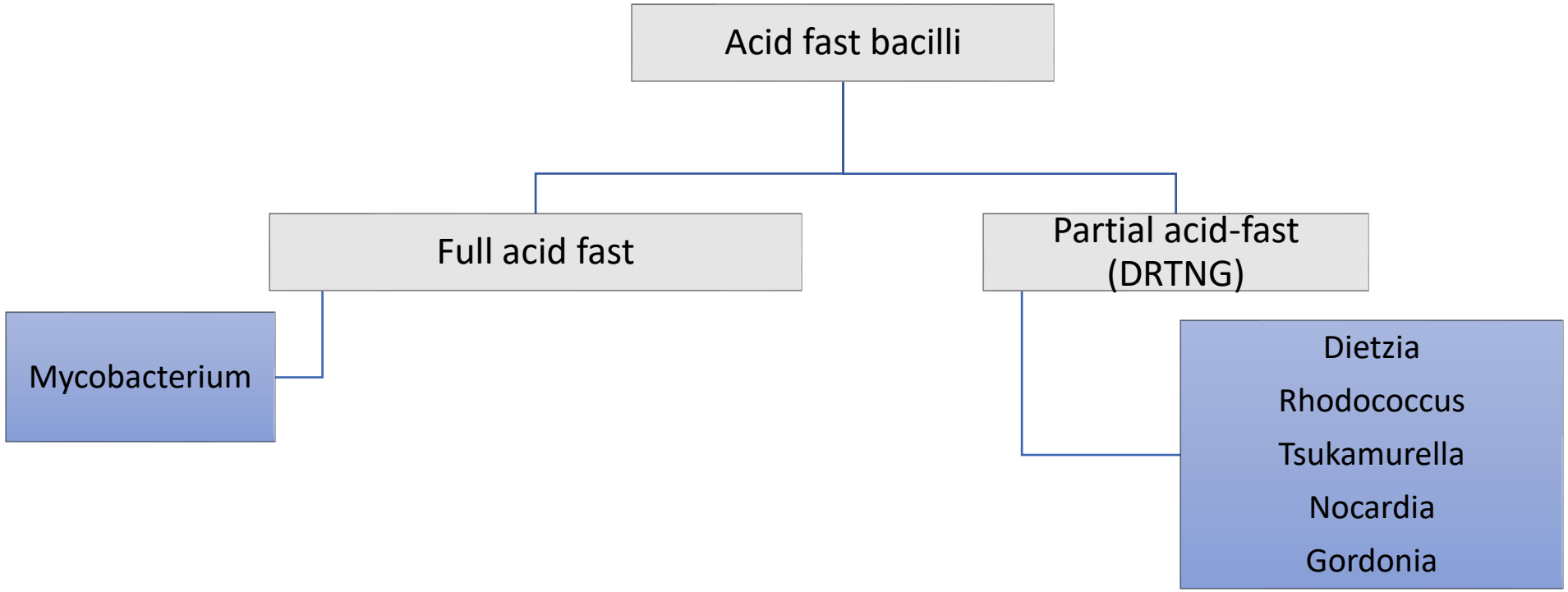
Others:

- Speaker's Bureau (Cepheid)
- Speaker's Honorarium (Open Access symposium) (Hologic)
- Honorarium speaker – Seegene/GenomeWeb

Agenda

- TB screening
- Biosafety notes
- Specimen processing
- Stain and direct NAAT testing
- Identification techniques – New and old
- Notable Mycobacterium species
- Susceptibility testing

Let’s start with the basics



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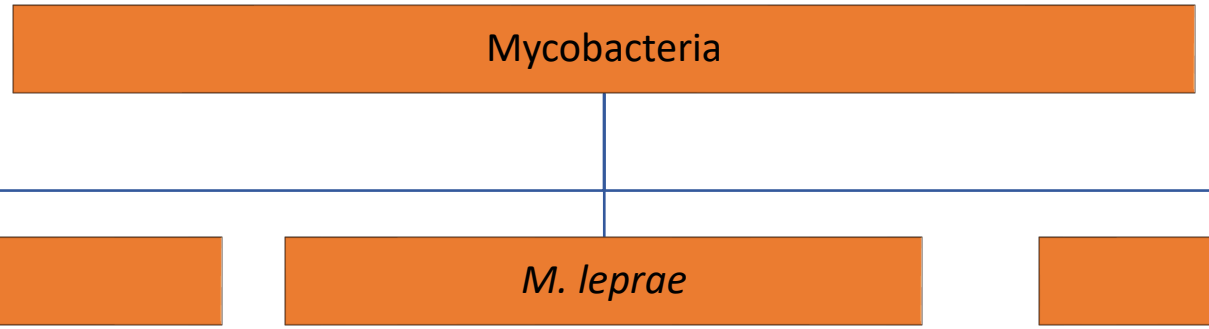
Mycobacteria species classification



*M. **t**uberculosis,*
*M. **b**ovis/ M. bovis BCG*
*M. **af**ricanum*
*M, **c**aprae*
*M. **m**icroti*
*M. **c**anettii*
*M **pi**nnipedii*
*M. **m**ungi*
*M. **o**rygis*

*0.01 to 0.03% synonymous nucleotide variation

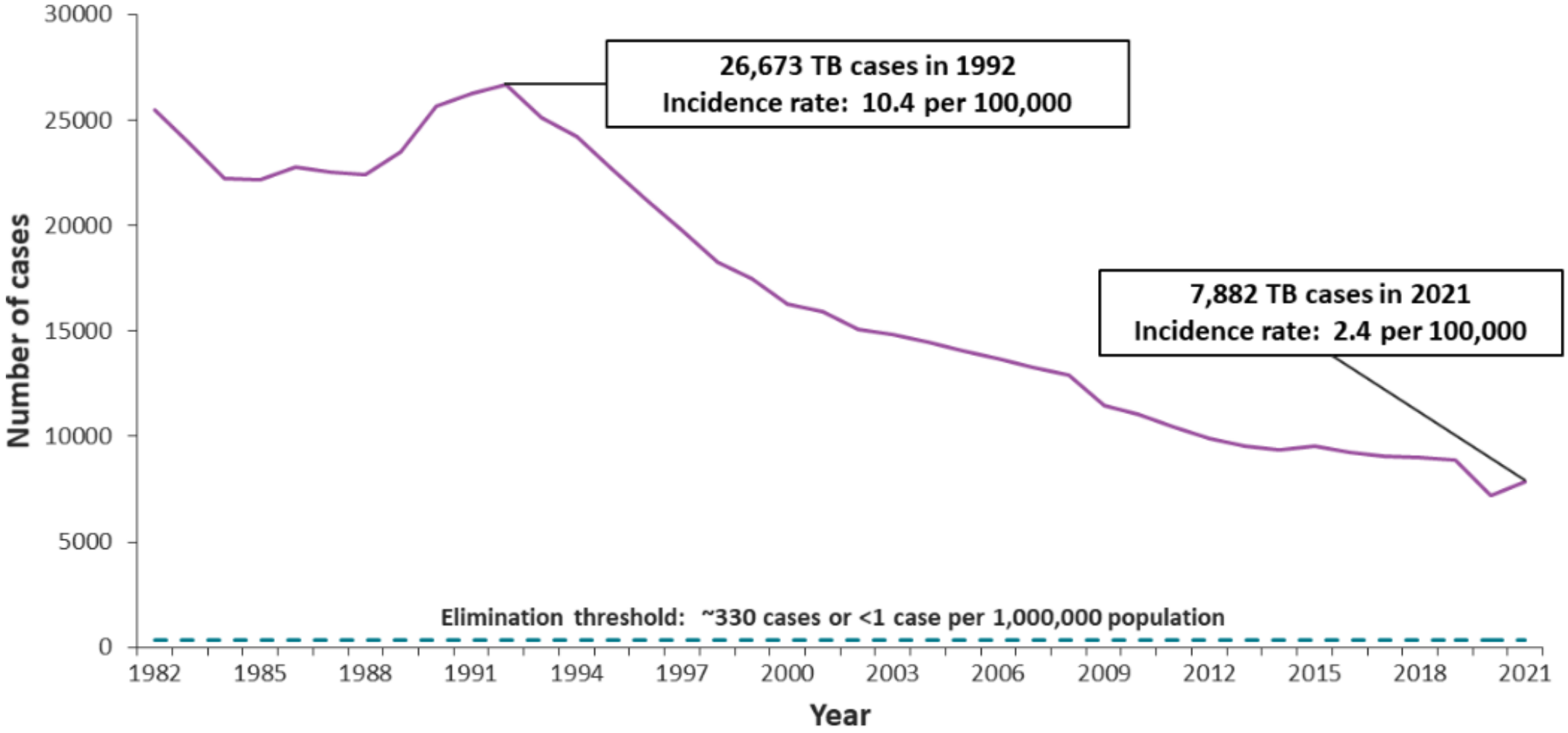
*no significant trace of genetic exchange among them

****ABC of MTBcomp**

Runyon Group	Characteristics	Species
I- Photochromogen	Pigmented production ONLY when exposed to UV/grown in light	M. kansasii M. marinum
II- Scotochromogen	Pigment production regardless of growing in dark or in light	M. gordonae M. scrofulaceum
III-Nonchromogen	Not pigmented when grown in the dark or in light	MAC M. asiaticum M. genavense M. haemophilum M. malmoense M. simiae M. szulgai M. terrae M. ulcerans M. xenopi
IV- Rapid grower	Growth on solid media in ≤7 days	M. fortuitum C M. chelonae M. abscessus M. smegmatis M. mucogenicum

Modified from Dr. Dona Woji

Resurgence of MTB during the pandemic



CDC

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TB screening overview

A brief overview on IGRA (Interferon gamma release assay) testing

- Up until 2001, TST (tuberculin skin test) is the only available test – LTBI and active TB

- TST vs. IGRA: Injection of purified Tuberculin derived protein (PPD); TST: same antigen derived from BCG
- Cannot distinguish from active and remote infections
- Two FDA-cleared commercial products for IGRA: Quantiferon (Qiagen) and T-spot (Oxford Immunotech)

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IGRA – Quantiferon TB

- **<u>QuantiFERON-TB Gold Plus (QFT-Plus) test</u>** is the fourth generation Interferon Gamma Release Assay (IGRA).
- Assessing cell-mediated response through a quantitative measurement of IFN-γ (ELISA) in a whole blood sample.
- QFT-Plus has two distinct TB antigen tubes:
- Both the TB1 tube and TB2 tubes - peptides from ESAT-6 and CFP-10 → elicit CMI responses from CD4+ T-helper lymphocytes;
- TB2 tube - additional set of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes.
  - TB1-Nil >0.35 is interpreted as positive
  - TB2- Nil > 0.35 → POS
  - TB2- TB1 difference, reflecting CD8 activity (particularly if >0.6 iu)- the more likely new infection /active disease
  - Indeterminate results : any results >8 Nil tube , <0.5 Mitogen Tube

Mitogen – Positive Control
Low response may indicate inability to generate IFN-γ

Nil – Negative Control
Adjusts for background IFN-γ

TB1 – Primarily detects CD4 T cell response

TB2 – Optimized for detection of CD4 and CD8 T cell responses

Nil	TB1 - Nil	TB2 - Nil	Mitogen - Nil	Qualitative Result	Interpretation
≤8.0 IU/mL	≥0.35 and ≥25% of Nil	Any	Any	Positive	*M. tuberculosis* infection likely
	Any	≥0.35 IU/mL and ≥25% of Nil			
	<0.35 IU/mL OR ≥0.35 IU/mL and <25% of Nil		≥0.5 IU/mL	Negative	*M. tuberculosis* infection NOT likely
			<0.5 IU/mL	Indeterminate	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0 IU/mL	Any				

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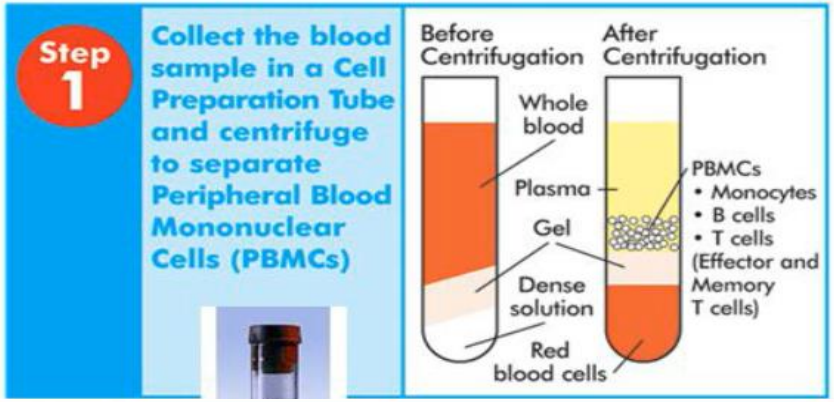
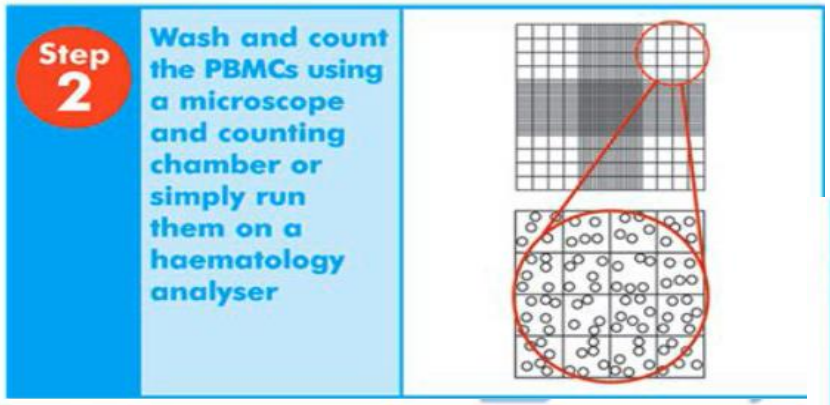
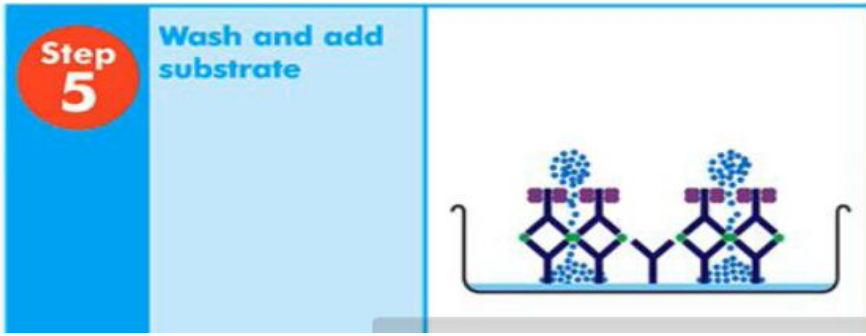
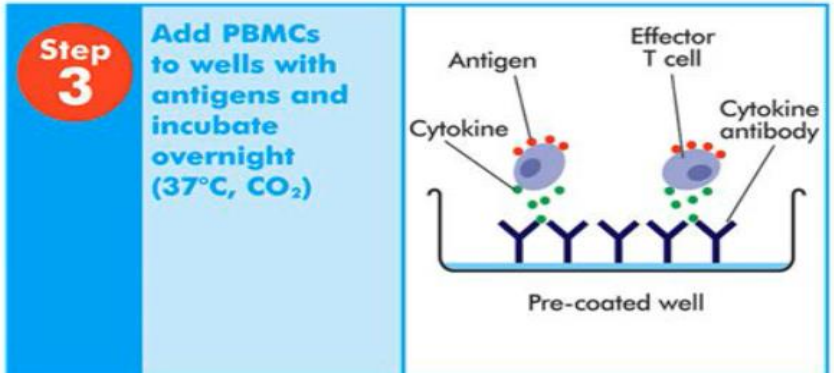
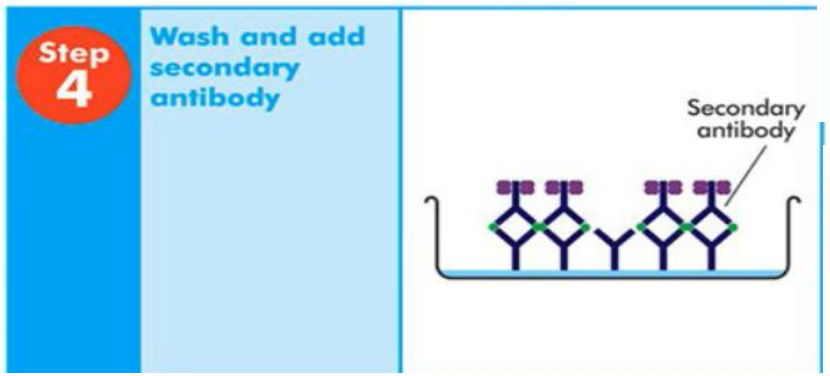
T-SPOT®.TB test (T-Spot)

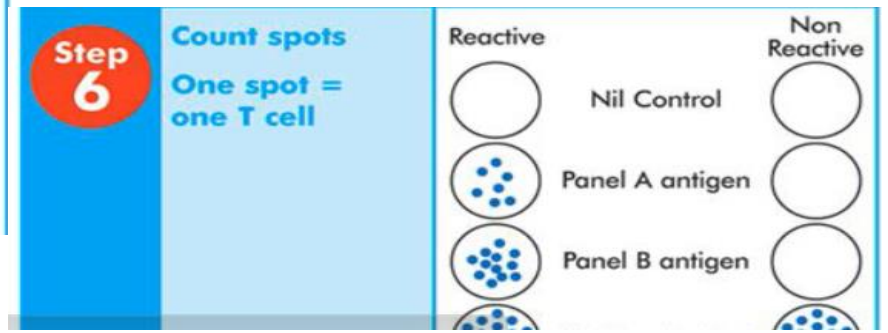












Interpretation Criteria for T-SPOT.TB Test (T-Spot)

Interpretation	Nil*	TB Response†	Mitogen§ (Positive Control)
Positive¶	≤10 spots	≥8 spots	Any number of spots
Borderline**	≤10 spots	5, 6, or 7 spots	Any number of spots
Negative††	≤10 spots	≤4 spots	≥ 20 spots
Indeterminate**	>10 spots	Any	Any number of spots
	≤10 spots	<5 spots	< 20 spots

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Which one is better? QFT or T-spot?

Table1: Differences in Currently Available IGRAs

	QFT-GIT	T-Spot
Initial Process	Process whole blood within 16 hours	Process peripheral blood mononuclear cells (PBMCs) within 8 hours, or if T-Cell Xtend® is used, within 30 hours
***M. tuberculosis* Antigen**	Single mixture of synthetic peptides representing ESAT-6, CFP-10 & TB7.7.	Separate mixtures of synthetic peptides representing ESAT-6 & CFP-10
Measurement	IFN-g concentration	Number of IFN-g producing cells (spots)
Possible Results	Positive, negative, indeterminate	Positive, negative, borderline, invalid

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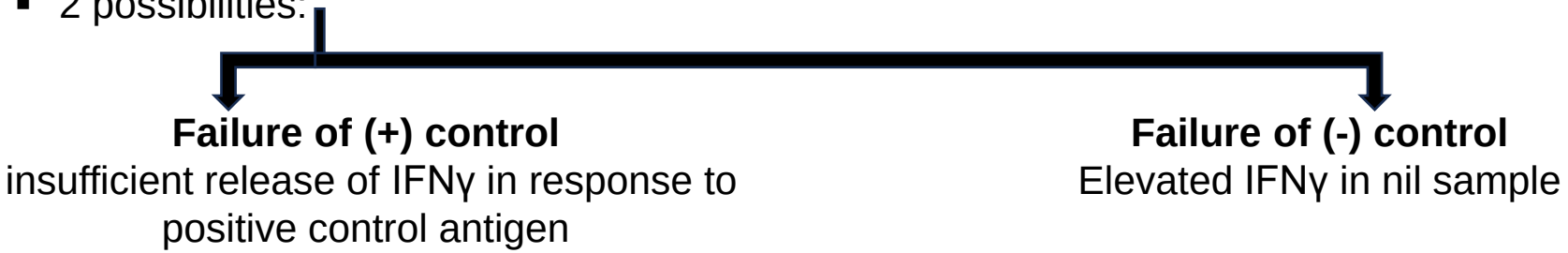
Limitations

- **False positive:**
  - *M. szulgaii, M. kansasii, and M. marinum* : expression of ESAT-6 and CFP-10
- **Children**:
  - IDSA: TST preferred over IGRA for < 5 years of age, although use in those > 3 years of age may be appropriate
  - AAP (12/2021):
    - For < 2 years of age: TST recommended, IGRA acceptable
    - Consider risk and BCG vaccination history
- **Indeterminate**:
  - Causes
    - Technical factors (delay in incubation, handling of blood specimen)
    - Immune status of individual (Mitogen insufficient response)

  - 2 possibilities:



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Modified from Dr. Helen Tsai

TST vs. IGRA

		IGRA		TST (2,4)
Single visit assay?	YES	Requires only one patient visit	NO	Requires two patient visits
Sensitivity?	YES	>94% sensitivity – accurately identify TB-infected patients	NO	70% sensitivity – more missed diagnoses
Specificity? (5)	YES	>97% specificity – less unnecessary follow-up and treatment	NO	Variable; 59% in BCG-vaccinated populations - false positives result in costly follow-up
Objective results?	YES	Objective and controlled laboratory assay	NO	Subjective measurement of skin induration
Affected by BCG vaccination?	YES	Unaffected by BCG vaccination	NO	Results affected by BCG vaccination
Most cost effective? (6,7)	YES	More cost effective than TST in multiple screening situations	NO	High program costs due to second visits and false positives

- ↑ chance of IGRA positivity – prevalence regions/countries
- + TST ➔ + IGRA ➔ healed TB, ↑ LTBI /high risk of pulmonary d/s
- + TST ➔ Neg IGRA ➔ low risk of developing pulmonary d/s
- IGRA – higher specificity

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Chawla H et al. Infect Public Health. 2012 Dec; PMID: 23287606.

- Questions so far?

What is the infectious dose required for M.Tb airborne transmission?

A) 1-10 organisms
B) 25 organisms
C) 35 organisms
D) 100 organisms

What is the infectious dose required for M.Tb airborne transmission?

A) 1-10 organisms

B) 25 organisms

C) 35 organisms

D) 100 organisms

A few biosafety NOTES

- MTB: Infectious dose **1-10** organisms – No safe level of exposure
- Airborne droplet nuclei can be spread through normal air currents for long periods of time and spread throughout a room or building
- Mycobacteria are able to survive for weeks to months on inanimate objects if protected from sunlight; Resistant to acids, alkali, and some chemical disinfectants than most other non-spore-forming bacteria; MUST USE EPA approved disinfecting agents effective against Mycobacteria
- **Risk Group/BSL**
  Risk Group 3 (WHO: high individual risk, low community risk)
  Biosafety Level 3 Practices

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What would you **immediately** do if you accidentally dropped the positive AFB LJ slant culture that cracked and broke open on the floor when you were putting away specimens into the incubator?

A) Run away and never come back

B) Notify your superior

C) Hold your breath for as much as possible, then make sure BS cabinets are on and centrifuges are turned off, then leave the area, by closing the door for at least 30 minutes.

What do you **immediately** do if you accidentally dropped the positive AFB LJ slant culture that cracked and broke open on the floor?

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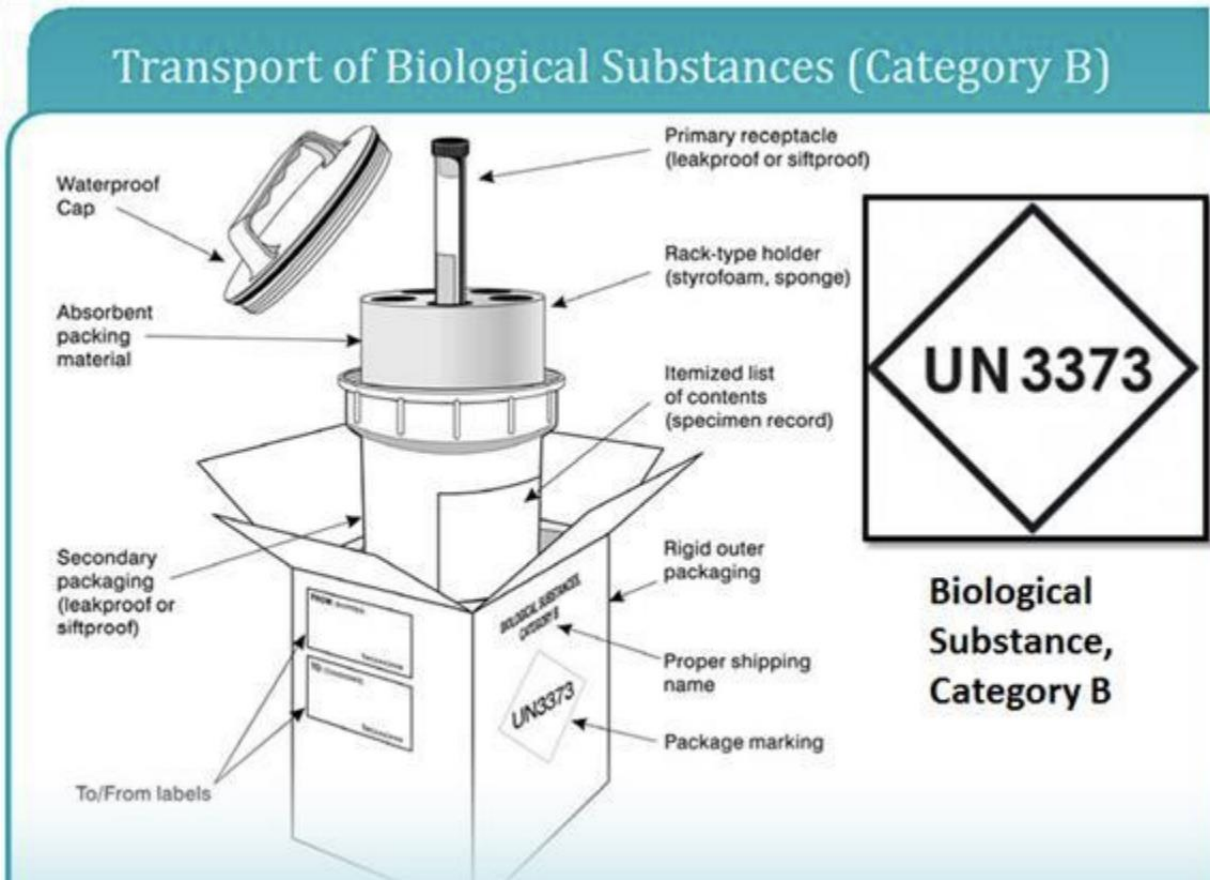
A few biosafety NOTES - continued

- All aerosols generating activity should be performed in class II Biosafety Hood
- NIOSH guidelines for respiratory
  - Requirement of respiratory procreative devices in the lab should be based on **risk assessment**
  - Person to person transmission occurs through exposure to patient with pulmonary symptoms and care of these patients should be performed **with N95 mask or PAP**R. Medical personnel are at risk when performing autopsies, intubation, bronchoscopies or by dermal inoculation procedures. Patients with suspected pulmonary tuberculosis should be placed in airborne isolation

Transportation and Transfer of Biological Agents

- Transport of Biological Substances
  - A leak-proof primary receptacle(s);
  - A leak-proof secondary packaging containing
  - Enough additional absorbent material shall be used to absorb all fluid in case of breakage
  - For cold transportation conditions, ice or dry ice shall be placed outside the secondary receptacle. Wet ice shall be placed in a leak- proof container.
  - An outer packaging of adequate strength for its capacity, mass and intended use.

MTBC isolates (broth or solid) – Cat A
Patient specimens – Cat B



- Individuals must be **Certified** for packaging & shipping of category B infectious disease substances

Packing and Shipping Dangerous Goods: What the Laboratory Staff Must Know

Learn what the laboratory professional should know to be certified (or recertified) to ship Division 6.2 Materials.

2 hr Intermediate Available for Syndication

Slide modified from Dr.

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Specimens for AFB

Specimen type, Collection, Processing

Specimen collection – rejection criteria for AFB culture sampling

When do specimens get rejected? (check all that applies)

A) Mis-Labeled specimens (Identification does not match on requisition)
B) Cerebrospinal fluid (tube #3) collected in the early morning
C) Dried red-topped swabs
D) Pooled urine from 3 individual urine specimens (collected 8 hours apart from each other, with the first one collected in the morning)
E) Pooled sputum
F) Lung tissue received in formalin
G) Sputum left at room temperature for more than 24 hours
H) Stool left at room temperature for more than 24 hours
I) 3 Bronchial lavage specimens collected 8 hours apart from each other, with the first one collected in the morning

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* Specimen rejection criteria used for routine Respiratory culture should not be practiced for AFB smear & culture test order (eg. Rejection based on >25 squamous epi cells)

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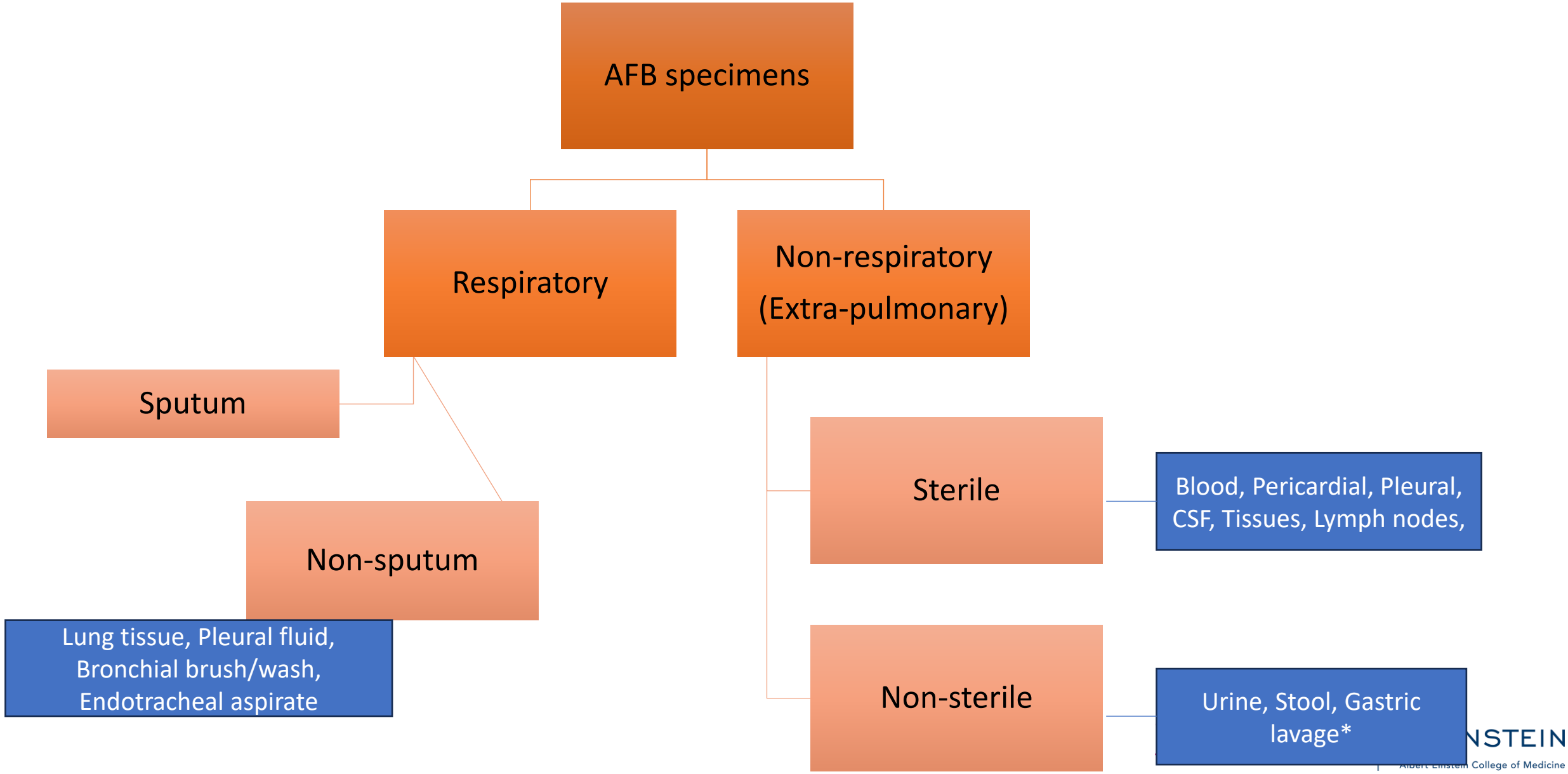
Specimens for AFB smear and culture



Specimen collection – types and requirements

- Pulmonary specimens – Sputum (induced, expectorated, deep cough)

Specimen from Normally Sterile Body Sites	Volume Requirements	Transport	Recommended for Isolation of MTBC?
Broncheoalveolar lavage (BAL)	minimum volume is 3 ml 10-15 ml is optimal	As soon as possible at room temperature; If delayed, refrigerate	Collect in 50 mL conical tubes or other sterile container (or sputum trap)
Bronchial washing or brushing			
Endotracheal aspirate			
Transtracheal aspirate			

Modified from APHL specimen processing

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Specimen collection – types and requirements

Specimen from Non- Sterile Body Sites	Recommended Collection Time	Volume Requirements	Collection Frequency	Transport	Recommended for Isolation of MTBC?
Gastric Aspirate	Early morning before patient eats and while still in bed	5–10 ml is optimal; maximum volume is 15 ml	One specimen per day on three consecutive ays	Room temperature; if delayed >1 hour, neutralize with 100 mg sodium carbonate	Yes
			ne specimen er day on hree onsecutive ays	If delayed >1 hour, refrigerate	Yes
			o ecommendatio	Refrigerate if delayed >1 hour, do not freeze	Mainly for diagnosis of disseminated MAC disease in patients with AIDS

Specimen from Normally Sterile Body Sites	Volume Requirements	Transport	Recommended for Isolation of MTBC?
Cerebral Spinal Fluid	10 ml is optimal; minimum volume is 2-3 ml	As soon as possible at room temperature; do not refrigerate	Usually paucibacillary; culture may have limited sensitivity
Other Body Fluids (pleural, peritoneal, pericardial, synovial)	10-15 ml is optimal; minimum volume is 10 ml	If delayed, refrigerate	Yes
Tissues or Lymph Nodes	As much as possible; add 2-3 ml sterile saline	As soon as possible at room temperature (no formalin, preservatives, or fixatives)	Yes
Blood	10ml preferred, minimum 5 ml. Collect in SPS or heparin tube, no EDTA	At room temperature, do not refrigerate or freeze	Mainly for diagnosis of disseminated MAC disease in patients with AIDS

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Mycobacteriology: Processing

Specimens received in lab
BLS-3 practices required (containment, PPE, BSC)

Sterile specimens

Eg: Extra-pulmonary specimens
CSF, Body fluid, Tissues, etc

Stain & Innoculate Culture

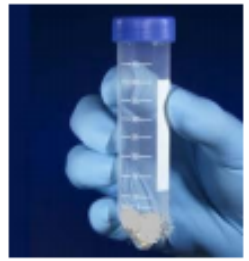
Non sterile specimens

Sputum ***
Stool, urine, BAL, gastric aspirate

Digestion (Breaking down mucus)

Decontamination (removing microbiota)

Centrifugation (Concentration)



Stain & Innoculate Culture

Classic Respiratory infection

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Let's talk about Specimen processing?

- What does specimen processing entail in setting up AFB smears and culture??

Specimens received in lab
BLS-3 practices required (containment, PPE, BSC)

Non sterile specimens

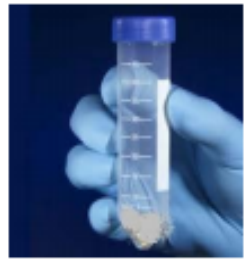
Digestion (Breaking down mucus)

Liquefaction
N-acetyl L-cysteine (NALC)
or DTT

Decontamination (removing microbiota)

2-4% NaOH

Centrifugation (Concentration)



Stain & Innoculate Culture

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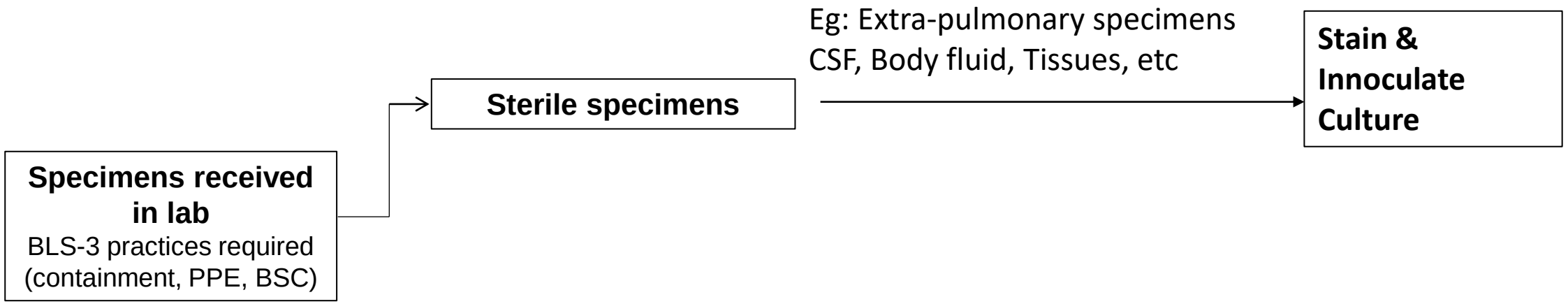
Processing sterile samples



- Normally Sterile Specimens
  - Normally sterile tissue samples may be ground in sterile 0.85% saline or 0.2@ bovine albumin and then inoculated directly into the media
  - Sterile Body Fluid – Concentrate by centrifuging at 3000 x g for 15 mins

What quality assessment should be routinely performed to ensure optimal recovery of mycobacteria from processing of non-sterile samples?

A) Monitoring % of thick mucousy sputum vs. liquidy (spit like) sputum received monthly

B) Monitoring % decontamination rate week or monthly

C) Assessing Proficiency testing failure rate

D) Assessing % competency of technologists performing AFB

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D) Assessing % competency of technologists performing AFB

CLSI recommendation:

$$\frac{\text{Number of cultures reported as } \textbf{“contaminated”}}{\text{Total number of cultures reported}}$$

Recommended level
3-5% Liquid only (7-8%) Solid (2-5%)

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Let’s have some fun with first case study

A Case of Ghost cells

- a 37-year-old African American woman with a history of poorly treated HIV infection, diagnosed in 2018, with progression to AIDS.
- A recent CD4 count was 7 cells / mL^3 with a viral load of 745,000 copies / mL.
- She presented to the ED with fever up to 102.3◦ F and a cough productive of yellow sputum with no hemoptysis. She denied any recent weight loss.
- She denied ever having a positive PPD but could not remember when she was last tested.
- A sputum sample was submitted to the microbiology laboratory for microscopy and bacterial and mycobacterial cultures. The Gram stain revealed a large number of leukocytes but no bacteria; several slender linear unstained structures were observed

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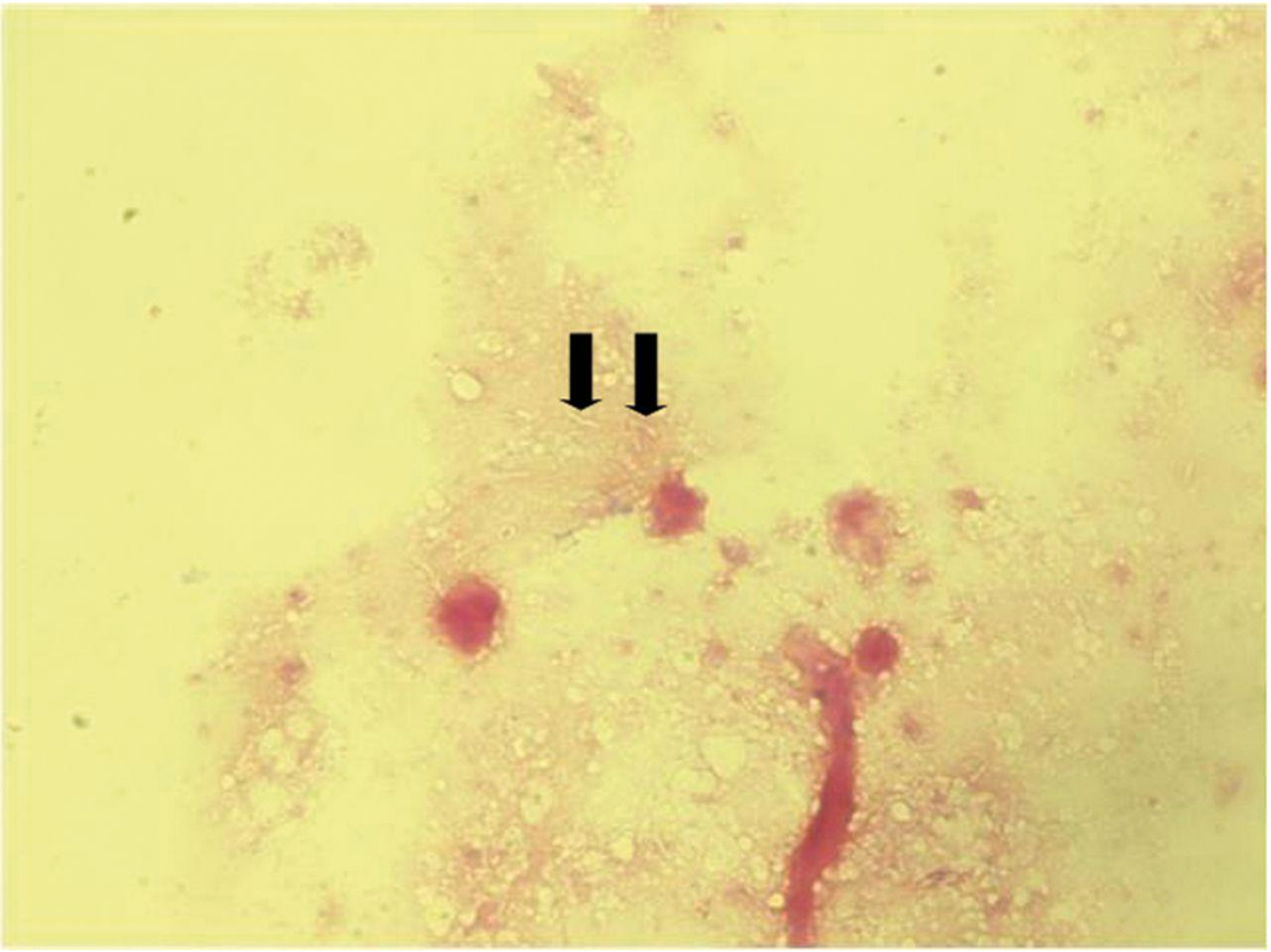
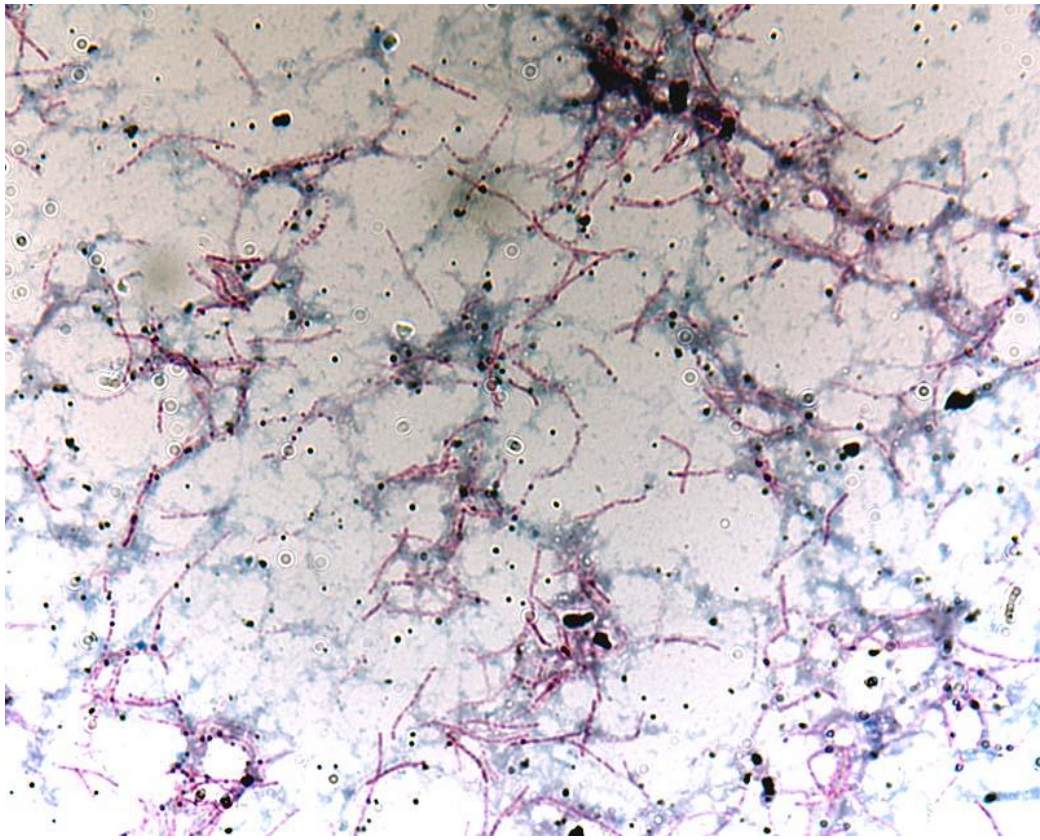
Case credit (Modified) to Dr. Walls and Pasculle, UPMC and JCM-Photo quiz (2013) Vol51, No4.

A Case of Ghost cells





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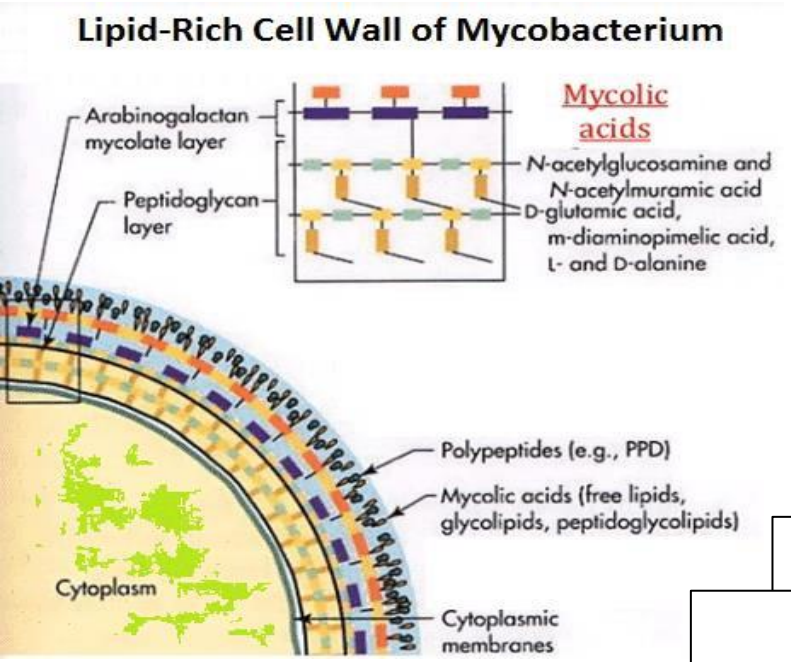
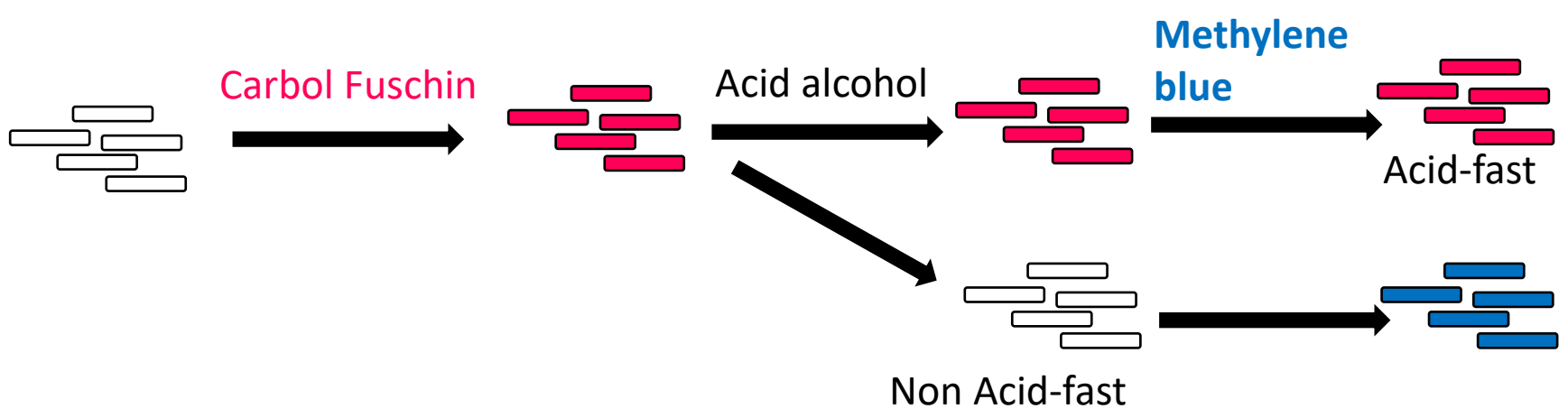
Case credit (Modified) to Dr. Walls and Pasculle, UPMC and JCM-Photo quiz (2013) Vol51, No4.

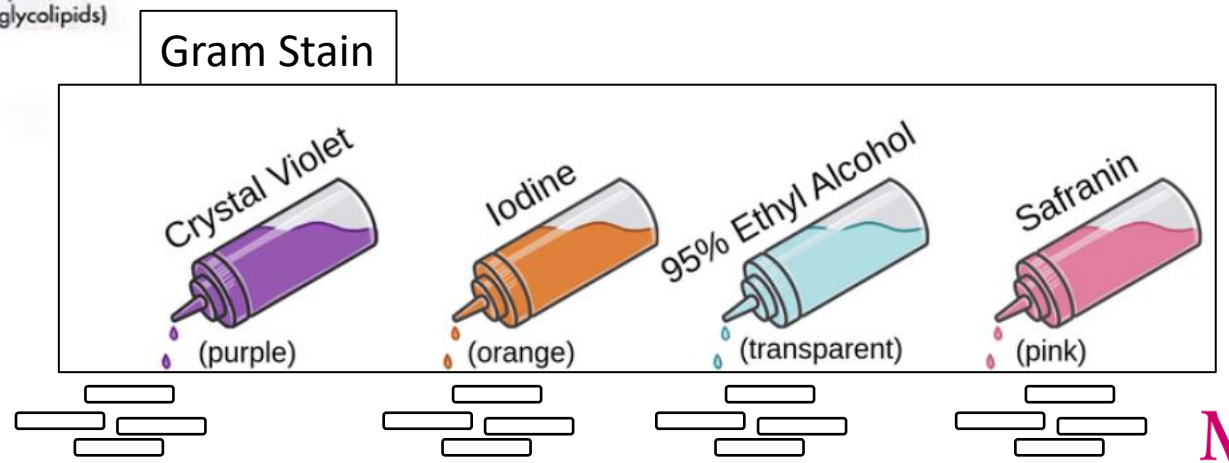
Why ghost cells?

Why Ghost cells on Gram Stain?

- Mycolic Acid in the cell wall can consist of 70-90 C molecules.







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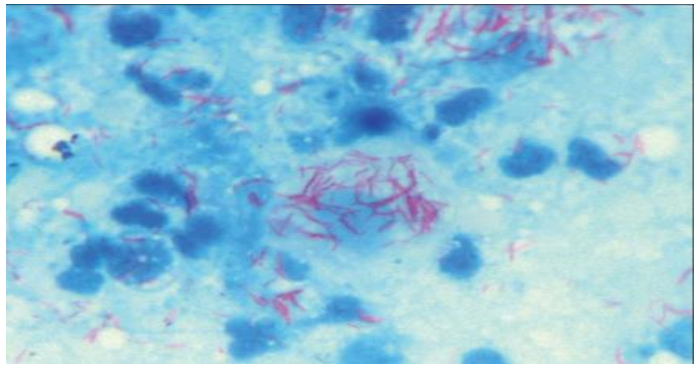
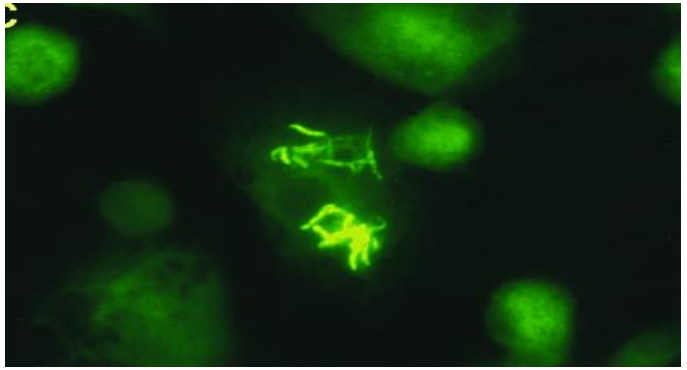
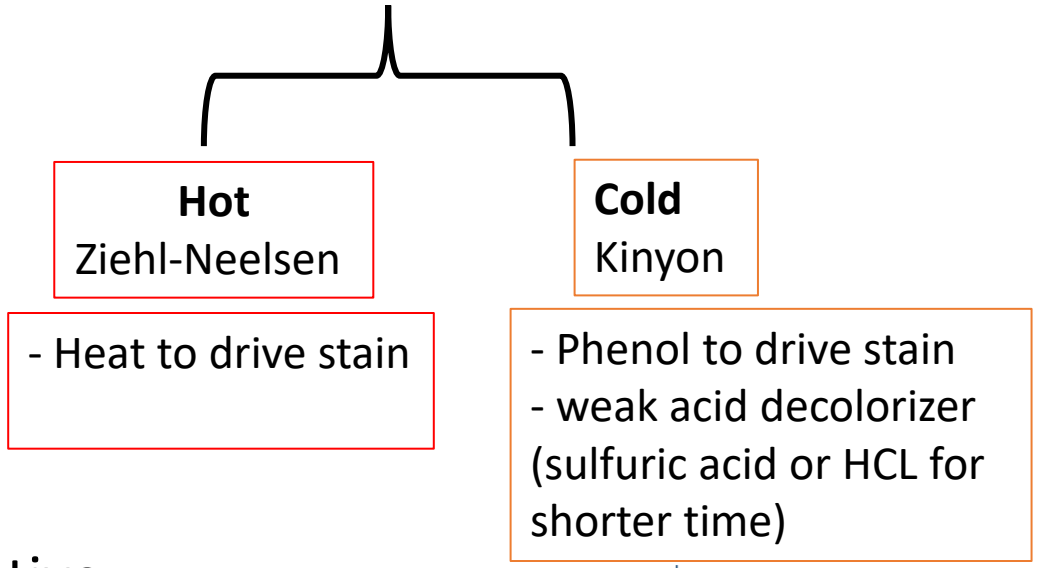
AFB Staining



- Fluorescent dye-based staining
  - Auramine O (followed by rhodamine)
  - Binds mycolic acid
  - Rhodamine – orange (gold color)



- Carbol Fuschin –based staining
  - Primary stain Fuschin forms complexes with mycolic acid



Report – semi quantitative (eg. 1+, 2+, 3+, 4+)

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Which stain is the most sensitive?

Lessons From a Proficiency Testing Event for Acid-Fast Microscopy*

Ákos Somoskövi, MD, PhD, Jacquelin E. Hotaling, BS, Marie Fitzgerald, Dianne O'Donnell, BA, Linda M. Parsons, PhD, and **Max Salfinger, MD**

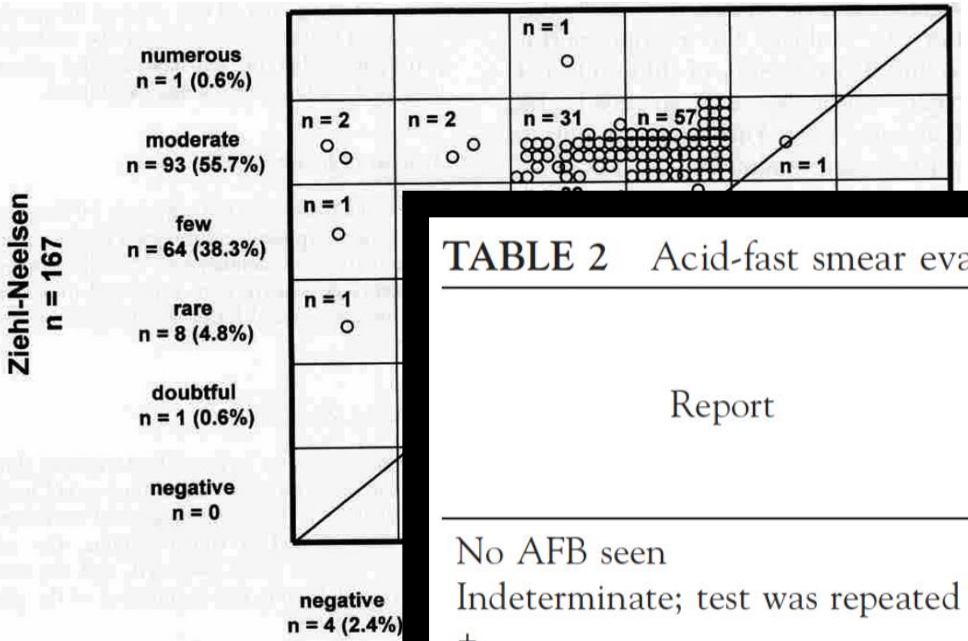

TABLE 2 Acid-fast smear evaluation and reporting[a]

Report	No. of AFB seen by the following staining method and at the indicated magnification, no. of microscope fields (no. of sweeps)[b]:		
	Fuchsin stain at ×1,000	Fluorochrome stain at:	
		×250	×450
No AFB seen	0	0	0
Indeterminate; test was repeated	1–2, 300 (3)	1–2, 30 (1)	1–2, 70 (1.5)
+	1–9, 100 (1)	1–9, 10	2–18, 50 (1)
++	1–9, 10	1–9	4–36, 10
+++	1–9	10–90	4–36
++++	>9	>90	>36

- Auramine-Rhodamine > ZN > Kinyon

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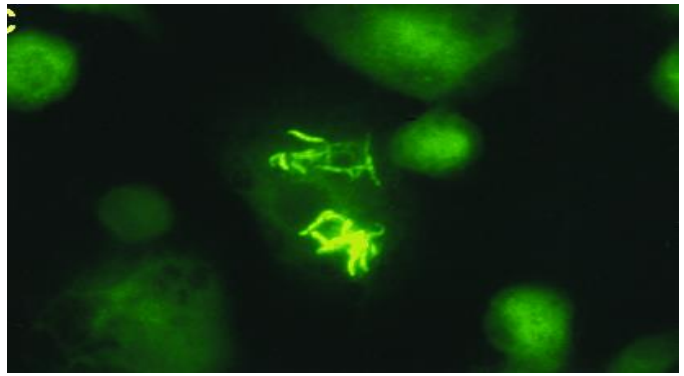
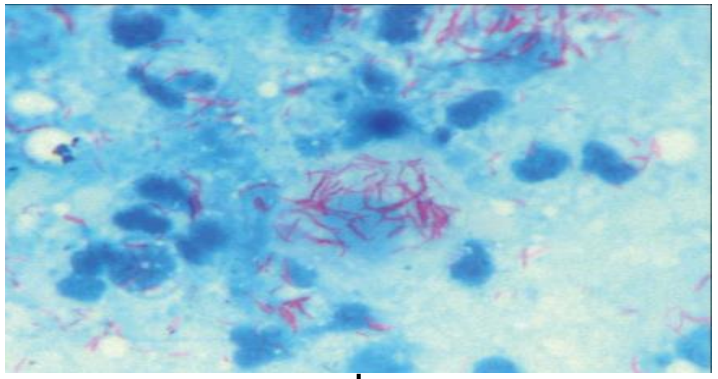
Somoskövi et al. Lessons from a proficiency testing event for acid-fast microscopy. JCM.

Applications of AFB stains – Fluorescent vs. Kinyon/Ziehl Neelsen stain





Use in

Direct clinical specimen after Digestion & Decontamination processing

Once MGIT (liquid) or solid culture media turns positive

Lower sensitivity with Non-tuberculous mycobacteria (NTM)

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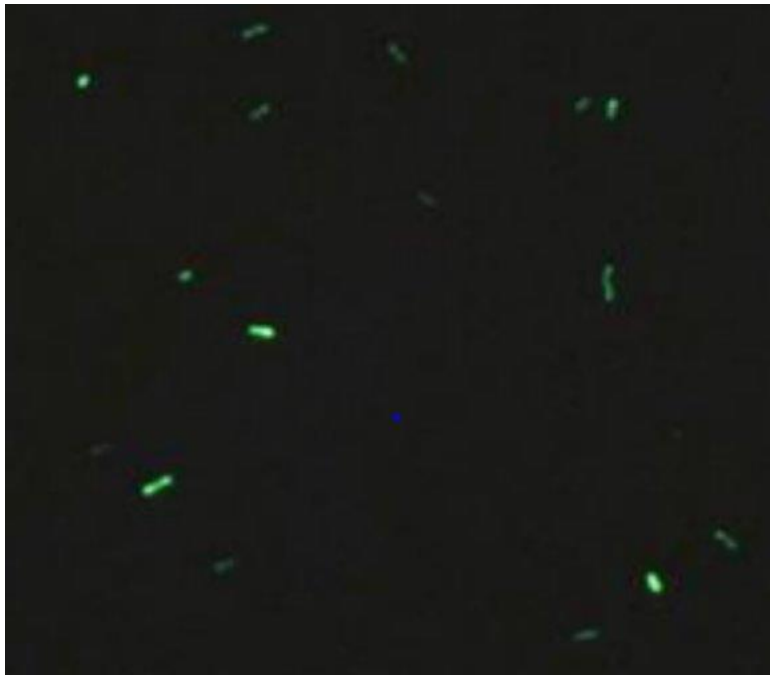
What is the AFB burden in sputum required for reliable detection of *M. tuberculosis* by **smear microscopy**?

A) 1 – 10 CFU/mL

B) 100 CFU/mL

C) 10,000 CFU/mL



credit to Dr. Helen Tsai

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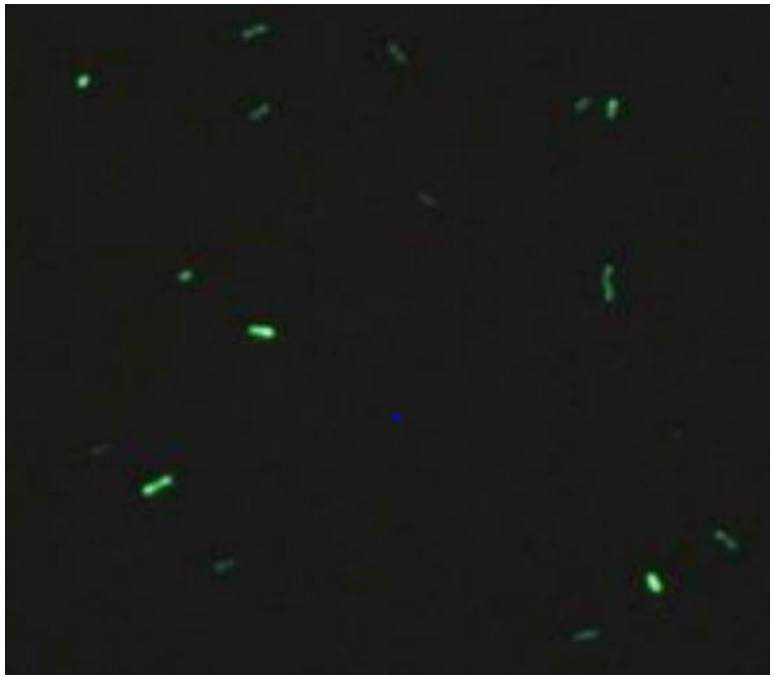

M. tuberculosis	Roche Cobas MTB
7.6 CFU/mL (sputum/BAL sediment)	
8.8 CFU/mL (raw sputum)	

How is it compared to NAAT testing from direct clinical specimens?

414 CFU/mL (Cepheid MTB PCR)

credit to Dr. Helen Tsai; PMID: 32656104

Use of NAAT-MTB testing from direct clinical specimens

*Non-FDA cleared (Ex-US)

	Manufacturer	Technology	Specimen type	TAT
COBAS MTB & MTB-Rif/INH*	Roche Diagnostics	Real-Time PCR	Sputum & BAL	2.5hr
Xpert MTB/RiF (Xpert MTB/RiF Ultra)*	Cepheid	Real-time PCR (molecular beacons)	Sputum (processed & unprocessed)	~ 2hr
TB-LAMP*	EikenChemical co	Loop-mediated isothermal amplification	Sputum BAL?	1hr
GenoType MTBDR *plus *(GenoType MTBDR*sl*)***	Hain Lifescience	Multiplex PCR + reverse hybridization	Sputum/BAL isolates	5hr
Abbott MTB & MTB RIF/NIH*	Abbott	Real-time PCR	Sputum & BAL	5-6hr
BD Max – MDR TB*	BD	Real-time PCR & Fluorogenic hybridization	Sputum (processed/un)	~3-4 hr

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Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia (Nathan). 2018. PMID: 29876241.

What is the criteria for removal of airborne isolation based on the direct AFB smear results?

A) 1 negative smear

B) 2 negative smears, from sputum samples collected 8 hours apart

C) 3 consecutive negative smears from sputum samples collected at least 8 hours apart

D) 2 negative smears from sputum samples collected 8 hours apart and 1 last negative smear from sample collected 3 hours after the second sample.

E) NO need, just based on the negative tuberculin skin test (TST) should be fine.

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Requirements for Airborne isolation

- APHL, WHO, and CDC guidelines
  - THREE good quality sputum, at least 8 hours apart – first morning good quality sputum

  Sputum – expectorated, deep cough, induced

  - THREE consecutive negative Sputum smears for removal of isolation … until early 2010's

Updates to the guidelines: Requirement for Airborne isolation

- 2016 APHL Consensus – TWO negative consecutive Xpert MTB/RiF
- 2020 -> WHO diagnostic → use of NAAT testing as SOC

- Sensitivity of smear microscopy - ~48% vs. MTB PCR: 96% (Culture as gold standard reference)

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Hamdi et al., J Clin Tuberc Other Mycobact Dis. 2020, PMID: 32923697

NAAT vs. smear sensitivity & specificity

Test	Specimen types	Methodology	Sensitivities (%)		Specificity (%)	
			AFB smear negative	AFB smear positive	AFB smear negative	AFB smear positive
Hologic Amplified MTD	AFB smear-positive and -negative concentrated sediments prepared from sputum, bronchial specimens, or tracheal aspirates	Transcription-mediated amplification of rRNA and detection by hybridization protection assay	64.0 for single specimen, 71.4 for 2 specimens	87.5 for single specimen, 100 for 2 specimens	99.1–100	100
Cepheid Xpert MTB/RIF	AFB smear-positive and smear-negative sputum samples or concentrated sediments prepared from sputum	Real-time PCR using molecular beacon probes to the 81-bp rifampin resistance-determining region of *rpoB*	73.1 for single specimen, 90.0 for 3 specimens	97.8 for single specimen, 99.5 for 3 specimens	97.9–99.0	97.9–99.0

Relatively high PPV with >1 specimen; High NPV

Workflow consideration for using direct PCR
- Smear pos only
Vs.
Smear neg only

Vs.
Any (concentrated sediments)

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Forbes et al. Practical guidance for clinical microbiology laboratories: mycobacteria. 2018. JCM

Interpretation of Initial AFB smear/NAAT results

CDC recommendation for interpretation of results on respiratory samples

AFB smear result	TB NAAT result	Interpretation
Positive	Positive	Presumed pulmonary TB Begin anti-TB therapy while awaiting culture and susceptibility results
Negative	Positive	Consider performing NAAT on a second specimen to confirm results Consistent with pulmonary TB but less infectious than AFB smear-positive TB Use clinical judgment regarding beginning anti-TB therapy
Positive	Negative	Rule out inhibition by using an internal amplification control Test additional specimen(s) by NAAT Consistent with the presence of NTM if all NAAT results are negative Use clinical judgment regarding beginning anti-TB therapy
Negative	Negative	Rule out inhibition by using an internal amplification control Multiple negative AFB smears and multiple negative NAAT results, in combination with other requirements, support discontinuation of airborne isolation Use clinical judgment regarding beginning anti-TB therapy

Direct NAAT test is not a replacement for routine AFB culture

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Use of rapid molecular test – CAP guidelines

COLLEGE of AMERICAN PATHOLOGISTS

- 2020 CAP implemented a new guideline

MIC.32150 Rapid Detection of *Mycobacterium tuberculosis* Complex - Laboratories Subject to US Regulations **Phase I**

A nucleic acid amplification test is available, either in the laboratory or by a referral laboratory, for the rapid detection of *Mycobacterium tuberculosis* complex on at least one respiratory specimen submitted to the laboratory (preferably the first diagnostic specimen) for mycobacteria culture.

- CDC & WHO algorithms for Dx of MTB highly recommends performing NAAT test on the initial respiratory specimen from patients suspected of having pulmonary TB.
  - Physicians request for high-indexed patients, who have signs and symptoms of pulm TB but diagnosis has not been established yet.
  - Those patients whose test results would alter case management or TB control activities

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Quality assessment – smear and NAAT

Indicator	Recommended Target	Numerator	Denominator
AFB smear	Within 24 hours of specimen receipt in the laboratory	Number of AFB smear results reported within target TAT	Total number of AFB smear results reported
NAAT	Within 48 hours of specimen collection	Number of NAAT results reported within target TAT	Total number of NAAT results reported
NAAT use[175]	Positive NAAT reported on 77% of culture-confirmed TB patients	Number of patients with positive NAAT results	Total number of culture-confirmed TB cases

CLSI. M48, 2018

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Identification of Mycobacteria

Culture methods

Identification methods

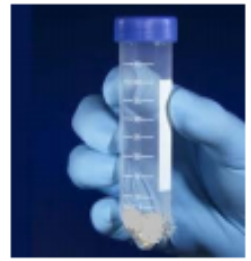
Getting to the culture growth

Specimens received in lab
BLS-3 practices required (containment, PPE, BSC)

Sterile specimens → **Stain & Innoculate Culture**

Non sterile specimens

- **Digestion (Breaking down mucus)**
- **Decontamination (removing microbiota)**
- **Centrifugation (Concentration)**



Stain & Innoculate Culture

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Liquid Culture Media

MGIT (Mycobacteria Growth Indicator Tube)

- Modified Middlebrook 7H9 broth base
- Growth → oxygen consumption → fluorescence of compound at the bottom tube tube
- Incubated in the **BacTec MGIT 960 System**
- Evaluated weekly for a total of **6** weeks (MGIT)
- Liquid broth media can allow for more rapid growth than solid media

VersaTREKculture system- pressure changes in the headspace*

MB/BacT Alert 3D (bioMérieux)- colorimetric CO_2 sensor

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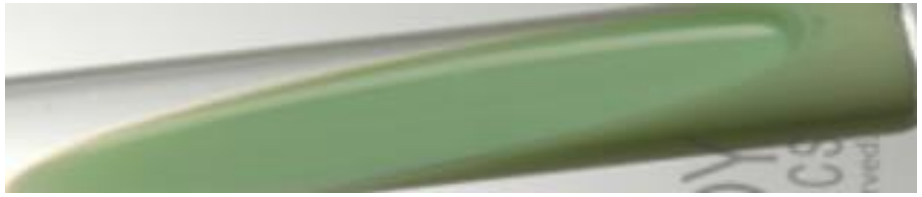
Credit to Dr. Helen Tsai for teaching slides

Solid Culture Media

Egg-based medium

Lowenstein Jensen Media (LJ-slant)

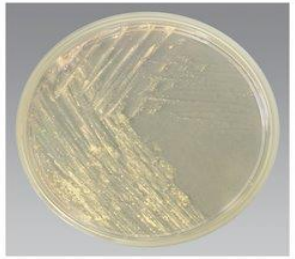
- Malachite green (dye that inhibits growth of most bacteria)
  - Prevents growth of contaminants that survived decontamination treatment
  - Encourages growth of *Mycobacteria*
- Incubated in a 5-10% CO2 incubator at 35 - 37°C
  - *(more on incubation temperature for certain NTM species next session)*
- Evaluated weekly for a total **8** weeks



Agar-based (Oleic acid) medium

7H9, 10, 7H11 plate or slant

- Synthetic oleic acid and 2% glycerol
- Suppress contaminants
- Faster growth than LJ
- Shorter shelf-life
- $$$



Credit to Dr. Helen Tsai for teaching slides(modified)

Culture Media

- Myco-F lytic media or BacTAlert or VersaTrek
  - Middlebrook 7H9 and Brain Heart Infusion broth formulation
  - Used for blood and bone marrow specimens (which are also inoculated on L-J media)
  - Incubated in BacTec instrument in main Bacteriology lab
  - 6 weeks of incubation

Modified from Dr. Bekah Martin

Recommendation of media use and incubation conditions

- Temperature: 35-37 ◦**C** (Most mycobacteria); 25-35 ◦**C** for samples from lower extremities (*M. hemophilum, M. marinum, M. ulcerans, M. chelonae)*
- Liquid media should be included for more rapid results/growth
- At least 2 media types for maximal recovery (CAP checklist: MIC .32250)
- Solid + Liquid media together
  - Maximized recovery
  - Colony morphology on solid
  - Mixed mycobacterial infections can be better detected
  - Solid can be backup whenever liquid media is contaminated (Eg; overgrowth of normal flora/insufficient decontamination)
  - Improved Recovery rate of 15% to 30% – adding liquid to solid

CLSI M48. 2018 & CAP checklist

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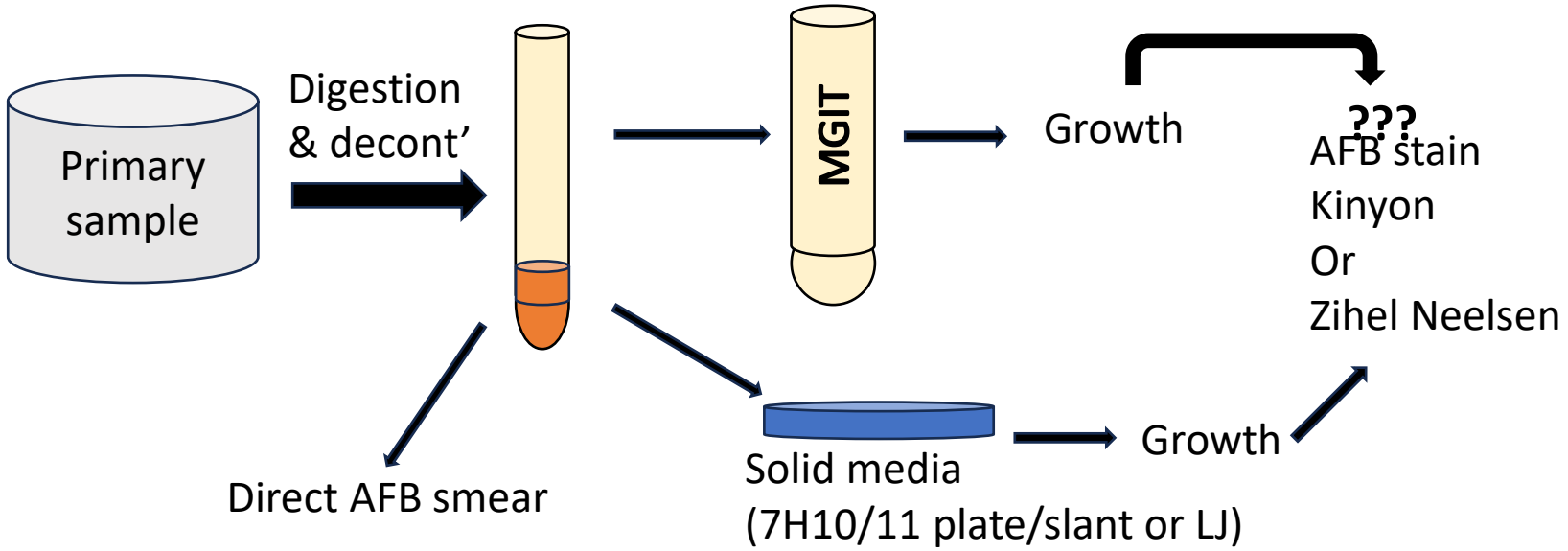
What do you do once a culture flagged positive?



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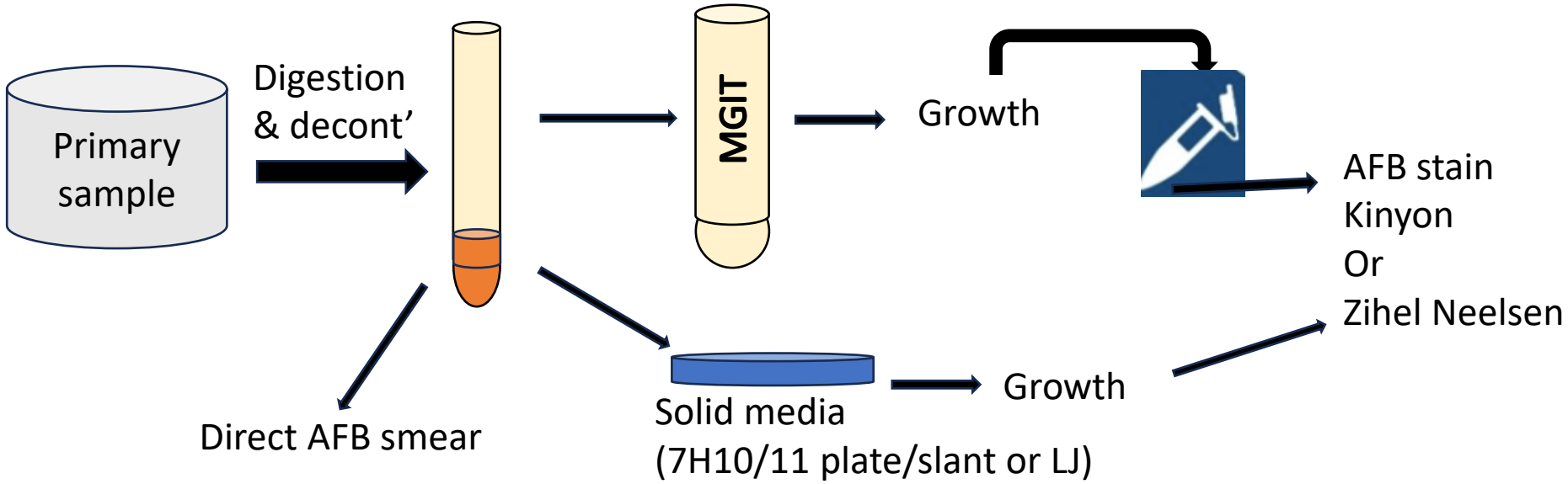

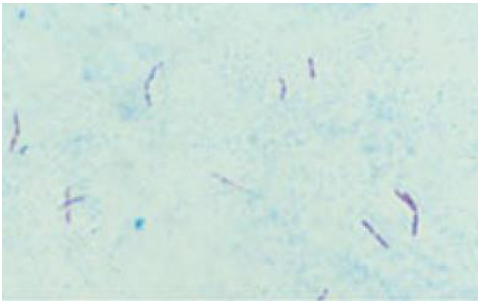

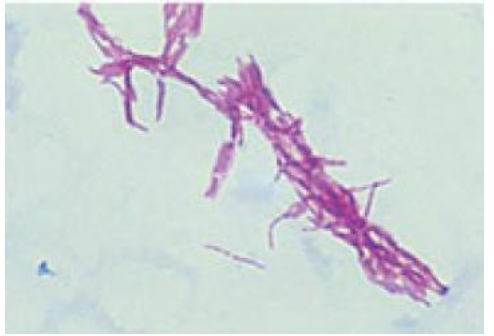

Koneman's Color Atlas of Microbiology, 7th edition

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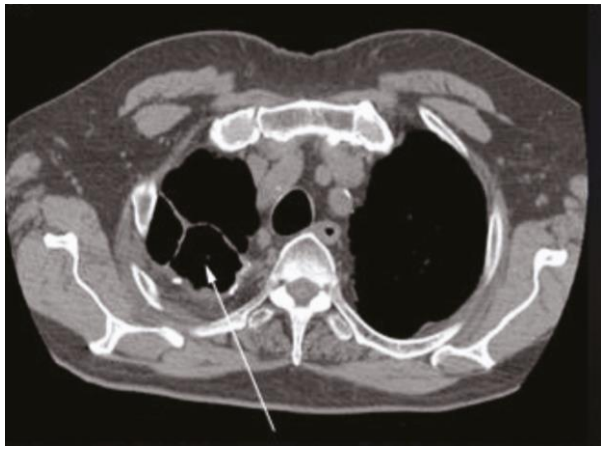
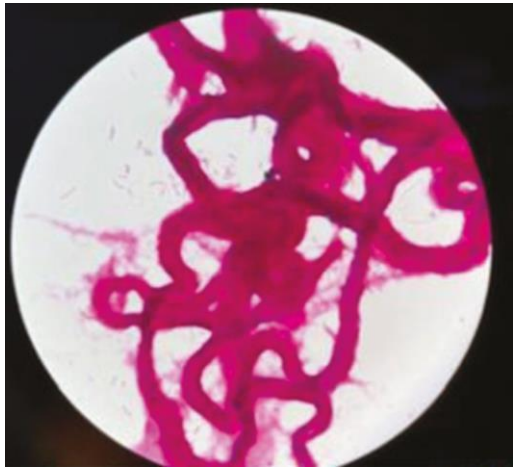
Case



- 79-yo male presented to pulmonary service w/ persistent productive cough ~2 months, fatigue, & night sweats.
- >30 years ago, asbestos exposure, COPD, emphysema, RUL cavitary lesion, HTN, & aortic aneurysm.
- Used O2 concentrator at home daytime & continuous pos airway pressure therapy at night.
- Worked on a shrimp farm until 8 yrs ago and no exposure to anyone with HIV
  - BAL → Cytology, AFB smear & culture, MTB PCR
  - MTB-PCR : Neg
  - Smear 1+ (only 1 out of 3 BAL)
  - All 3 MGIT cultures growth after 9 days of inc – Kinyon stain in pic
- CT scan- picture (newly developed nodular lesions)



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Marisa C Nielsen, Paula Clarner, Ping Ren, Wrong Place at the Wrong Maritime. CID. 2022

What is the cause of infection? And Why?

A) MTB
B) Non TB Mycobacteria
C) Co-infection with TB + Atypical mycobacteria
D) Non-infection related due to underlying COPD
E) Malaria

- MTB?
- Why: Fever, night sweats, cough for 2 months
- COPD, emphysema, etc.
- Growth ➔ +AFB; **** Cording** on Kinyon!!!**

- What about Neg MTB PCR??
  - BAL – not a claimed sample by Cepheid GeneXpert

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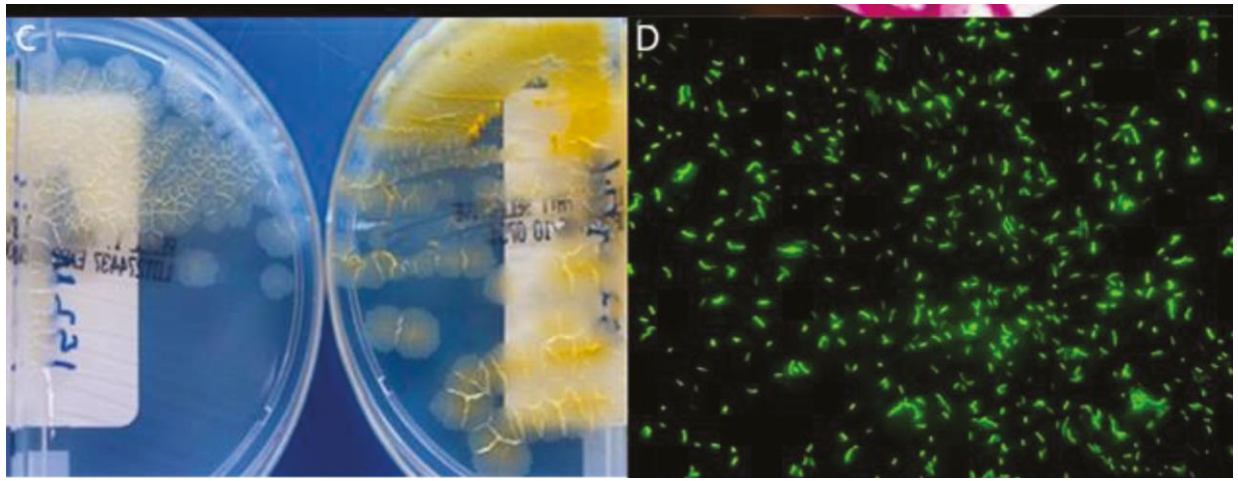
Case continued

- MGIT → DNA probe negative for TB
- Subculturing MGIT on 7H10 agar plate at 35C → pinpoint growth after 2 weeks
- Pinpoint colonies → MALDI → Identification
- Also subculture pin point colonies to 30C --> grew faster than at 35C and photochromogenic

- 2 months later, 2 expectorated sputum AFB culture showed same pattern; original smear 3+ AFB;



Now, what is the diagnosis?

Mycobacterium marinum

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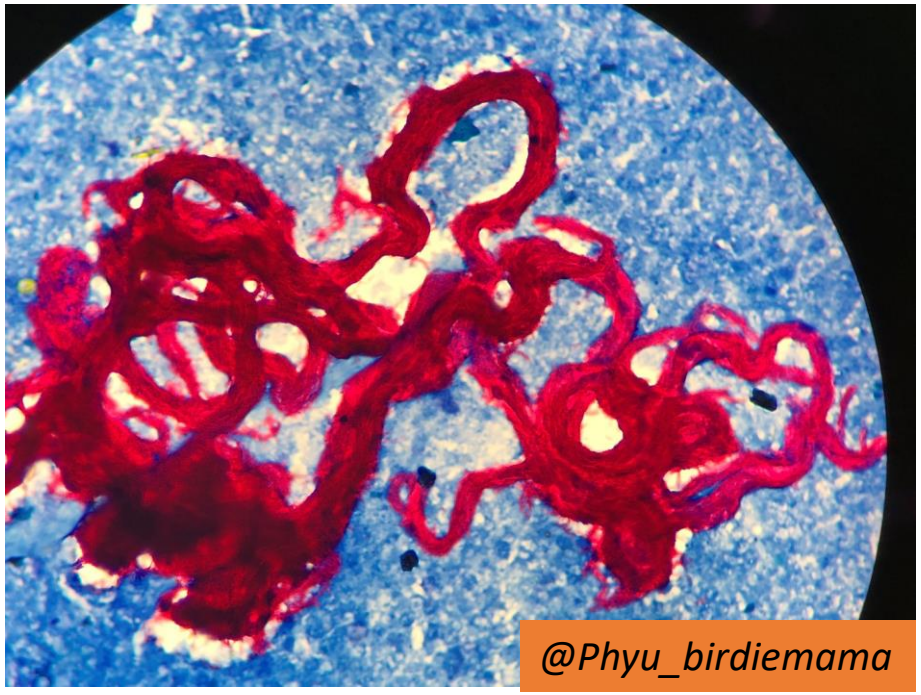
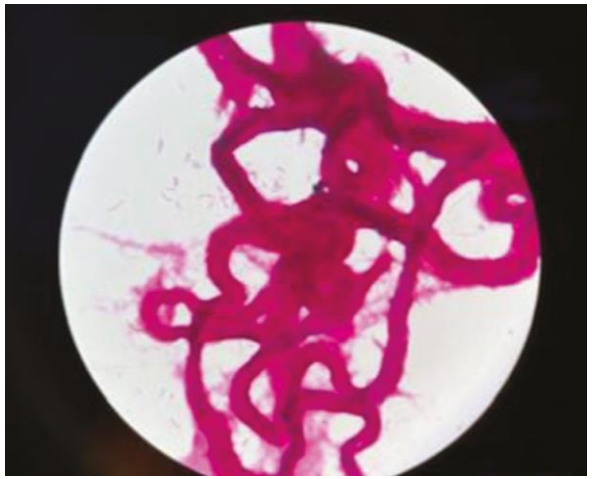
Marisa C Nielsen, Paula Clarner, Ping Ren, Wrong Place at the Wrong Maritime. CID. 2022

“A cording too cording” - Dr. Richard Davis





LABLOGATORY
A BLOG FOR MEDICAL LABORATORY PROFESSIONALS

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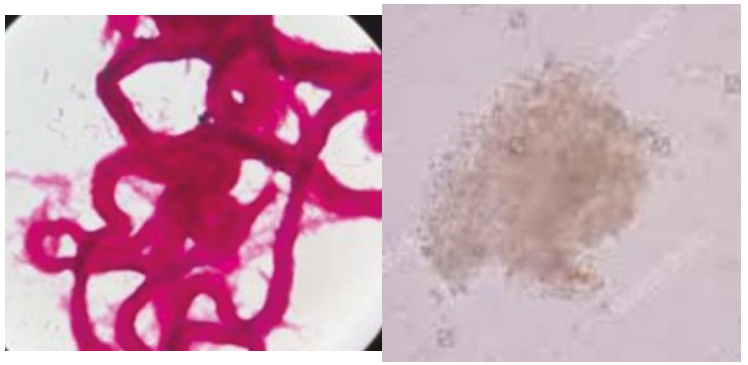
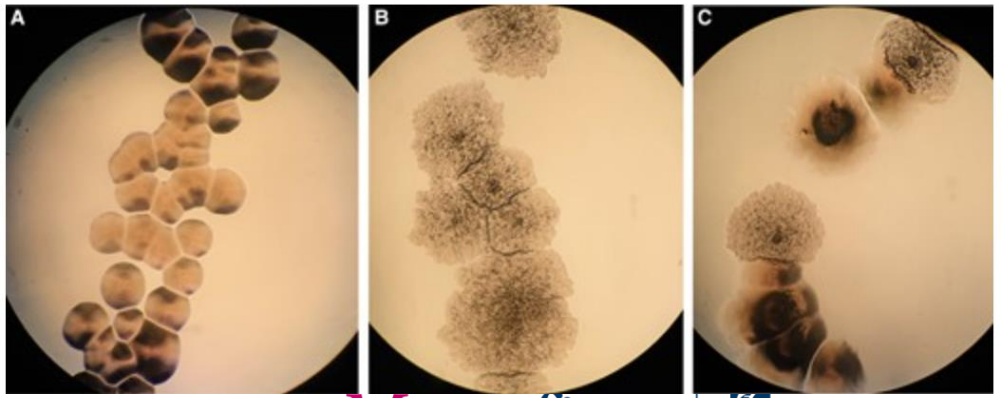
“Cord” factor

- “Cord” factor = serpentine cord appearance
- Highly enriched mycolic acid – trehalose 6,6 dimycolate (glycolipid) in Cell wall of mycobacterium
- Hallmark characteristics of Mtb – Cording from the beginning since Robert Koch

- Cording ≠ clumping
- Cording Mtb → rough colonies on solid agar
- Correlates with Virulence

- Cording in other mycobacterial species? (NTM?)
  - *M. abscessus, M. marinum*
  - Other Mycobacterium non TB species





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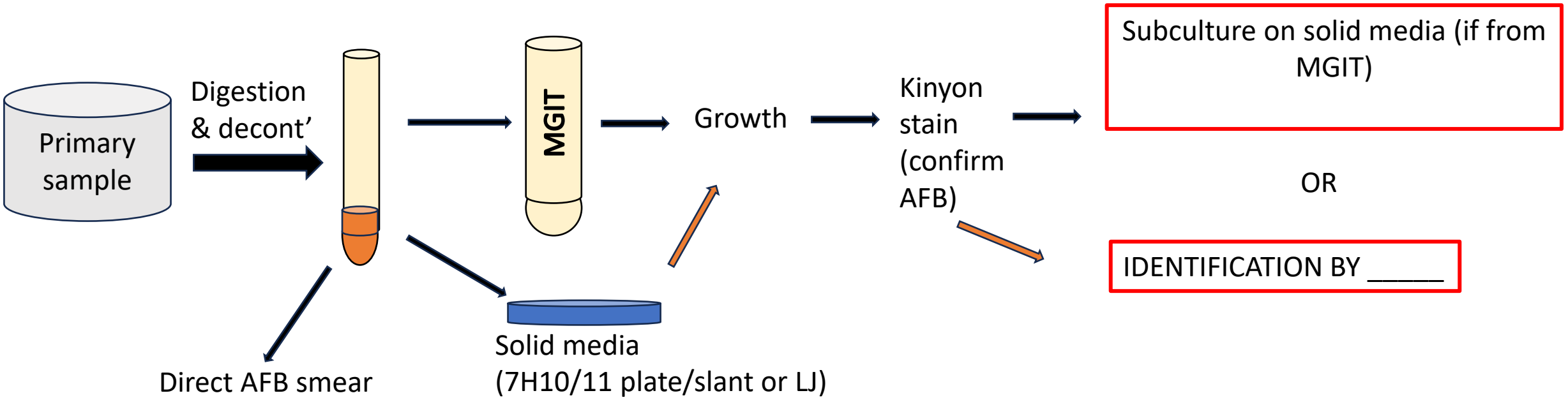
Wilhelm Hedin et al., JID, 2023
Lablogatory – a cording too cording by Richard Davis

Identification of Mycobacteria

Culture methods

Identification methods

Identification METHODS



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Out with the old

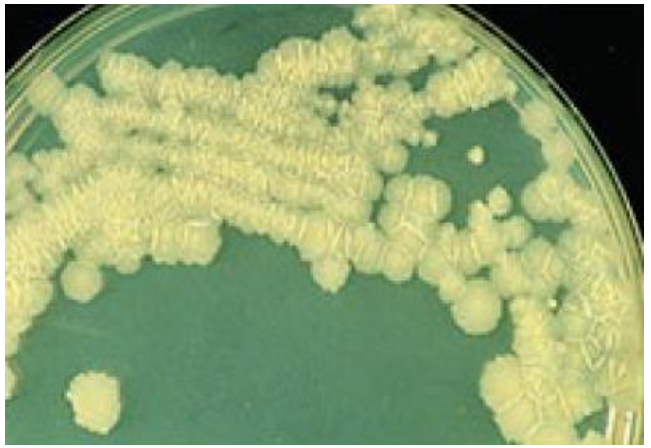
Growth rate and colony morphology

Biochemical-based method

- Nitrate reduction
- Niacin accumulation

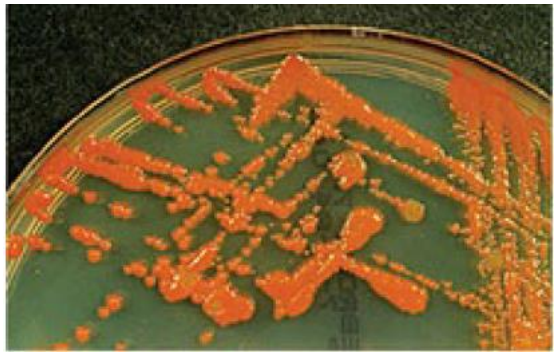
+ ➔ Mtb

(Some Mycobact produces Niacin; Mtb accumulates excess Niacin)



Mtb on Middlebrook 7H10

- Arylsulfate production
- Tween hydrolysis
- Urease (scrofulaceum + vs. gordonae =)
- Pyrizinamidase production



M. gordonae

- Require – sufficient growth
- Prolonged incubation time; not always reliable for all Mycobact species

Labmedicine blog
Koneman's Atlas; Manual of clinical Micro -11th edition

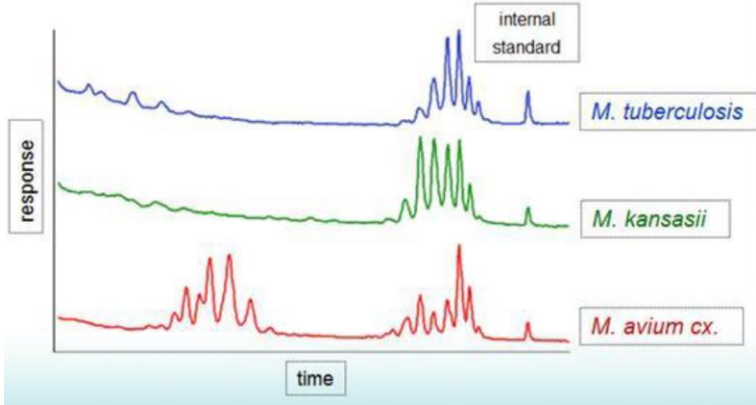
Out with the old - Biochemicals

TEST	UTILITY
Arylsulfatase	Helps distinguish pathogenic from non-pathogenic rapid growers; also useful for M. marinum, M. szulgai, M. xenopi, M. triviale.
68°C catalase	Helpful for identification of M. tuberculosis
Semiquantitative catalase	Helpful in certain circumstances. M. tuberculosis complex, MAC, M. xenopi, and a few other species produce <45 mm of bubbles.
Iron uptake	Helps distinguish M. chelonae from M. fortuitum.
MacConkey agar	Helps with identification of rapid growers.
5% NaCl	Helps with identification of rapid growers and M. triviale.
Niacin accumulation	Helps with identification of M. tuberculosis, M. simiae, some strains of M. bovis.
Nitrate reductase	Helpful in identifying many mycobacterial species.
Tween 80 hydrolysis	Helps distinguish some usually pathogenic from some usually non-pathogenic mycobacterial species.
Urease	Helpful in identifying many mycobacterial species.

High performance liquid chromatography (HPLC)

- Mycolic acid – extracted and UV absorbing or fluorescent esther – separated by chromatography (mass spectrometry)
- Unique peak Patterns generated as mycolic acids detected
- Separated by size /charge – pattern matched to a database /library of chromatograms
- Typically used in Public Health Laboratories

- Cost effective; identification of MTB and NTM (both from isolates and direct clinical samples)
- Equipment initial cost; potent reagents; RGM – problem identification



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In with the New/ Out with the Old – Accuprobe (Genprobe)

- Hybridization methodology – for ID of Mycobt from both solid and liquid media
  - Nucleic acid extracted → SS labeled DNA probes → anneal to target RNA
  - RNA –DNA hybridization detected by chemiluminescence

- Commercial kits for ID of

1) M tb
2) M. kansasii
3) M. gordonae
4) M. avium complex (MAC)

  - Product Discontinued

APHL; Genprobe

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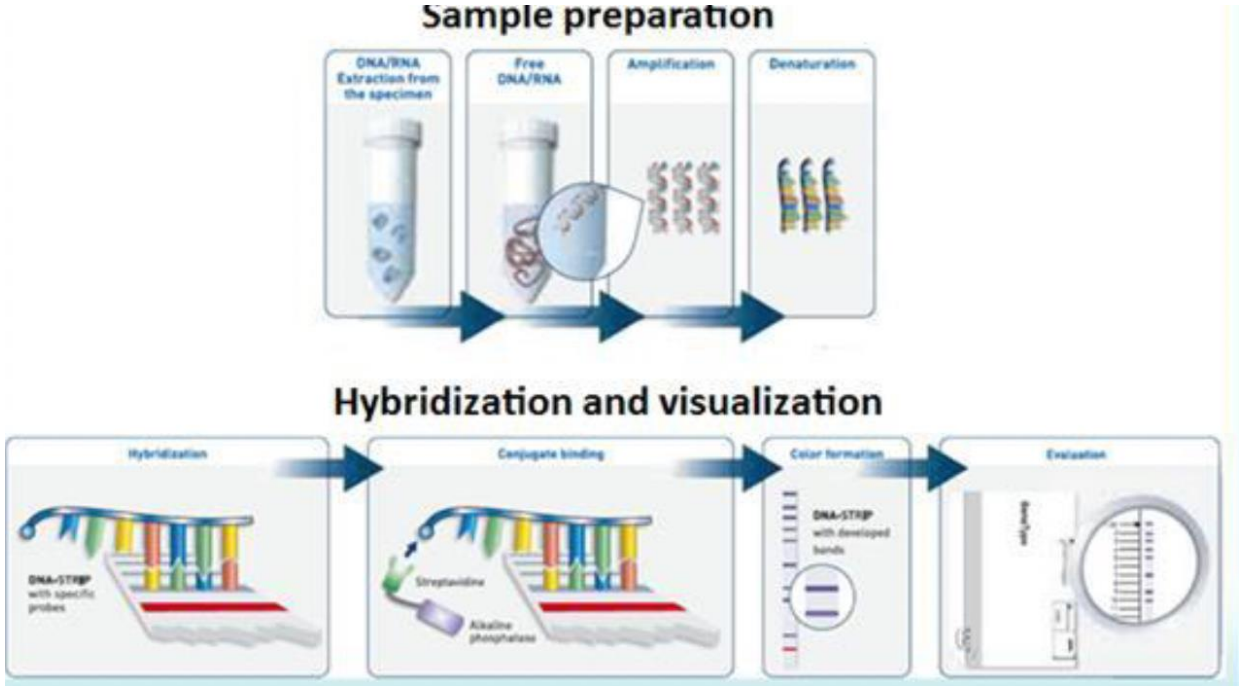
In With The New – Line probe assay (LPA)

- Nitrocellulose strips are used – reverse hybridization

DNA from lysed culture isolates hybridizes to probes and produces colorimetric bands when complementary DNA is present to allow for species identification



- Inexpensive; can detect drug resistance genes
- NON-FDA approved; Subjective interpretation d/t difficult to differentiate bands visually; sometimes identify certain species

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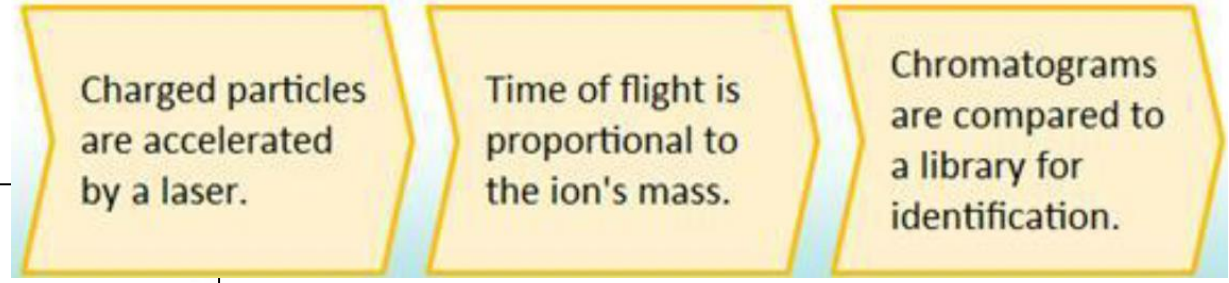
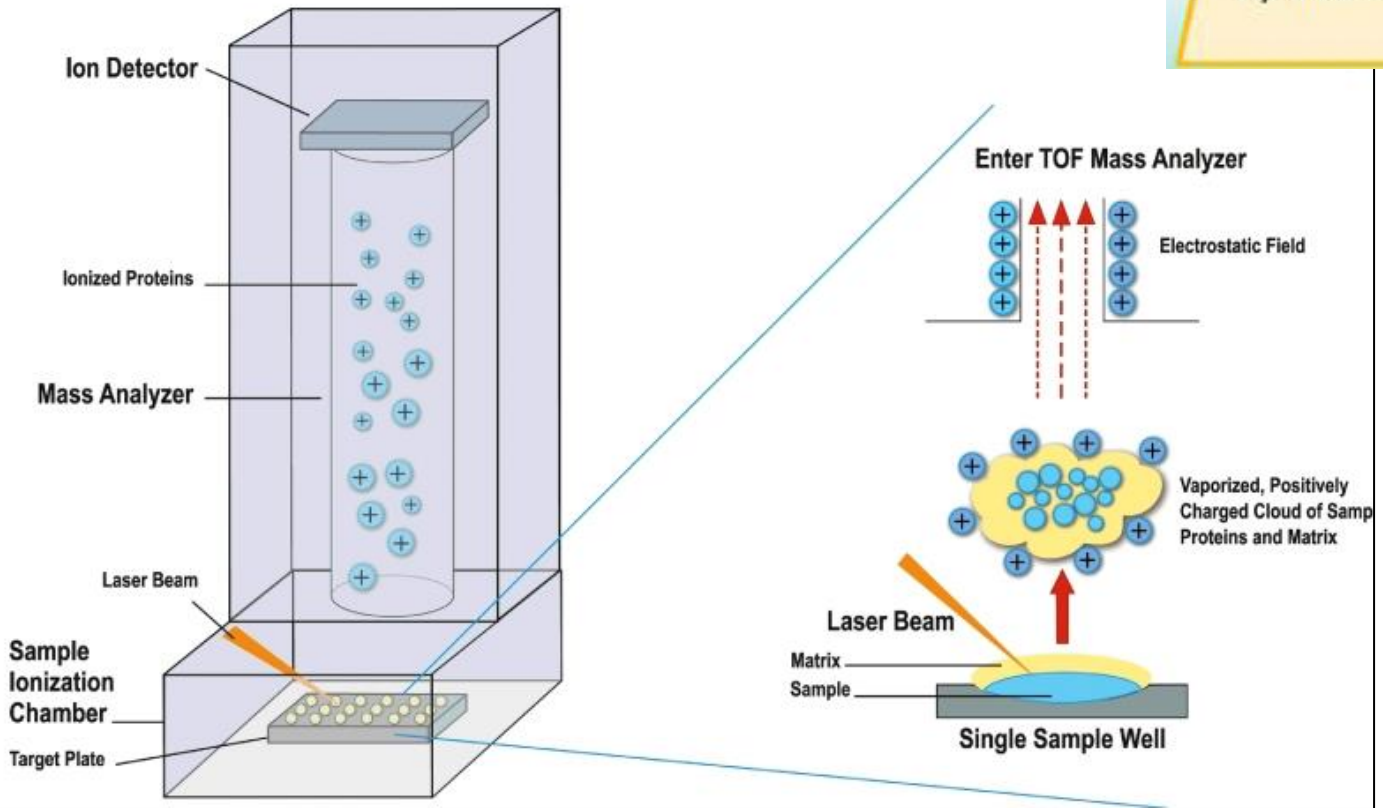
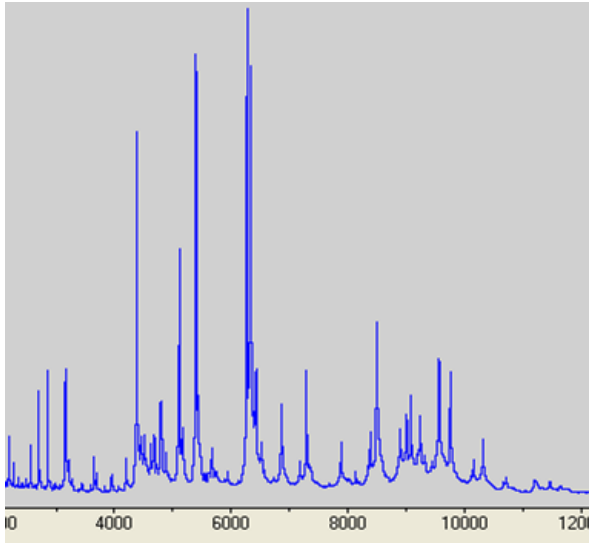
APHL; MCM

In with the New – MALDI-ToF

- “Matrix-Assisted Laser Desorption Ionization Time-of-Flight”
- Mass spectrometry –ased
- Proteins (peptide particles) – mass/charge







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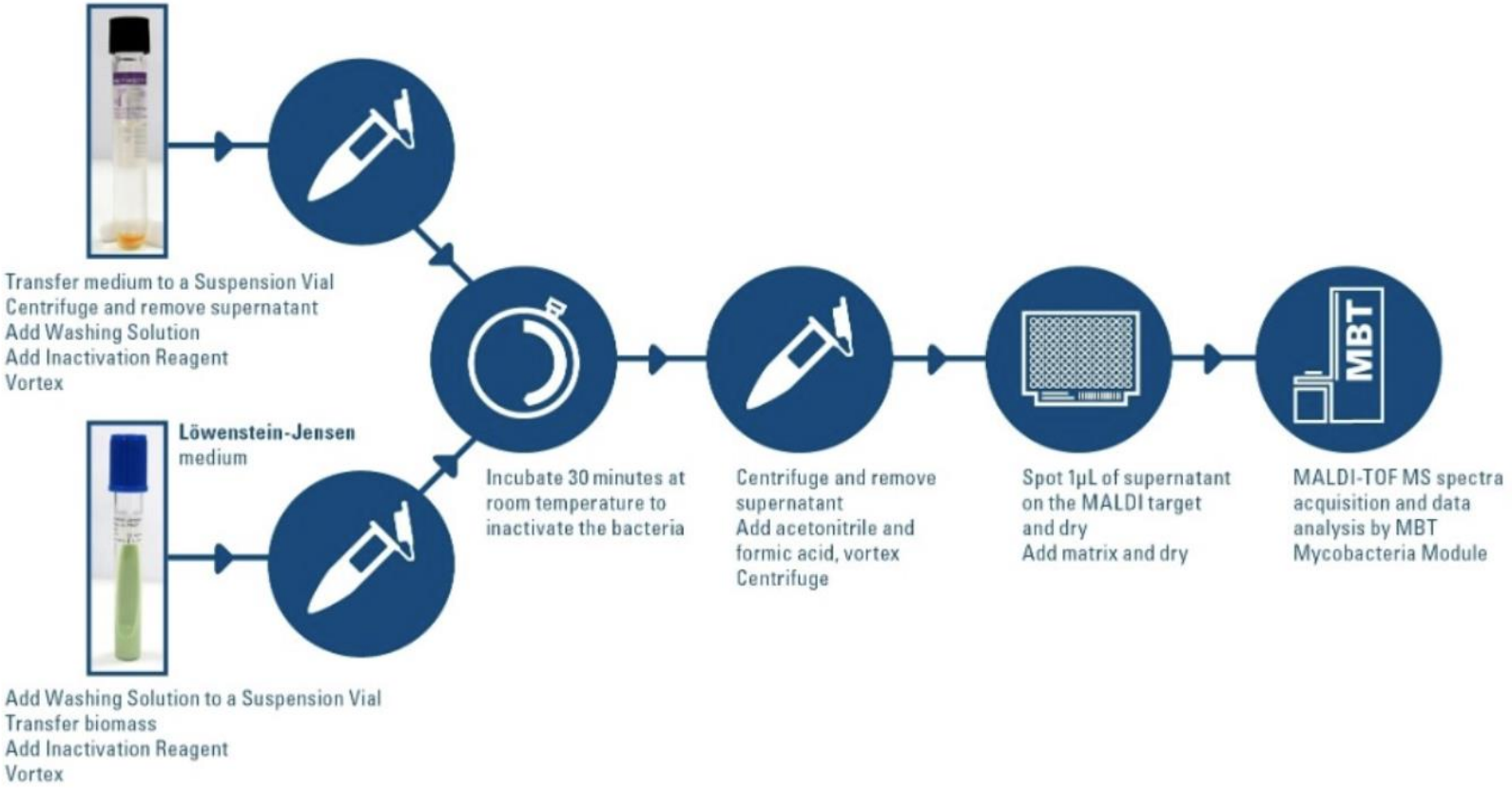
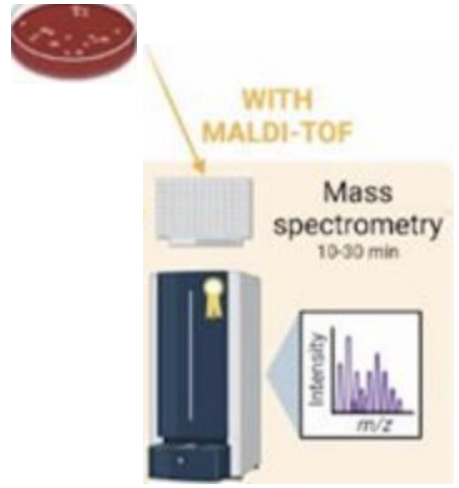
MALDI-ToF AFB – NOT just direct MALDI spotting!!!





Challenges with MALDI (liquid cx)

- Insufficient biomass
- Liquid broth – mixed growth of other organisms

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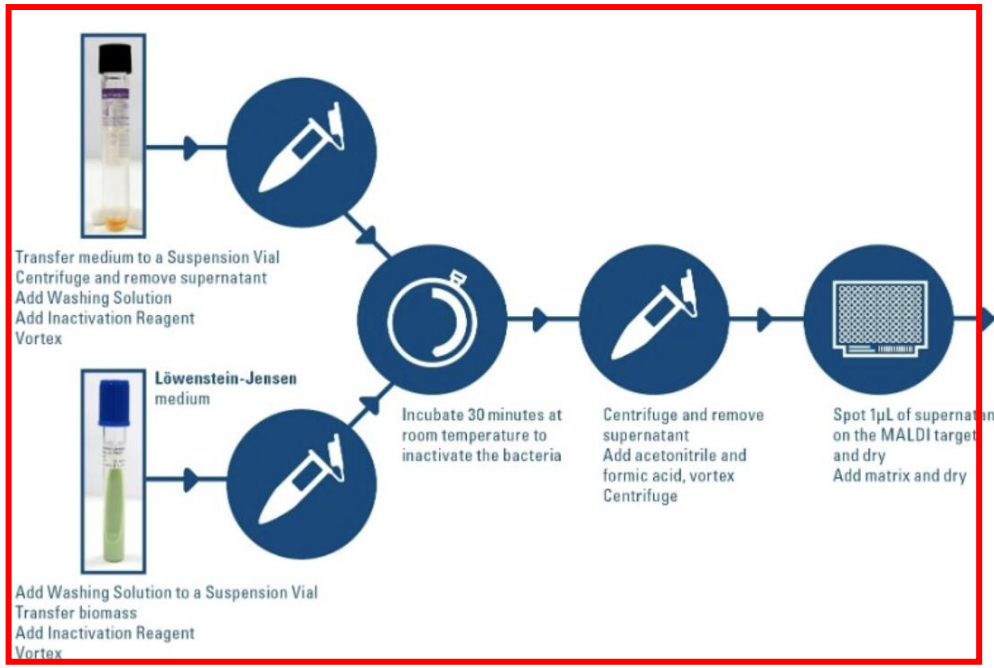
A safety note about using MALDI-ToF for AFB identification

- APHL (Association of Public Health Laboratory)
  : Safety assessment needs (routine basis)

What kind of safety assessment??

- Extraction/Inactivation process should be performed in BSC in BSL3
- Inactivation study – efficacy of inactivation procedure needs to be assessed periodically
  - Eg. 10 clinical and 1 reference strains from confirmed MTBC used monthly



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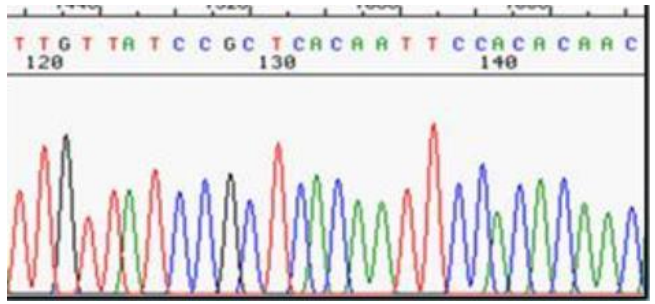
In with the NEW: Emerging Molecular methods

- NAAT (or Real-Time PCR)
  - Laboratory developed –target specific PCR NTM panels (reference laboratories?)
  - LPA – remember it's not a PCR
- Sequencing – 16s, rpoB. hsp65 genes (Sanger sequencing) or targeted Next Generation sequencing



- **Quicker TAT**
- **Targeted PCR/amplification → Positive liquid broth can be used (Potential – direct clinical specimens)**
- **Potential to recognize new strains**

- **Drawback: Cost cost cost!!!**
  - **Commercial test kits/platforms not widely available; May require as LDT (Laboratory Developed Test)**

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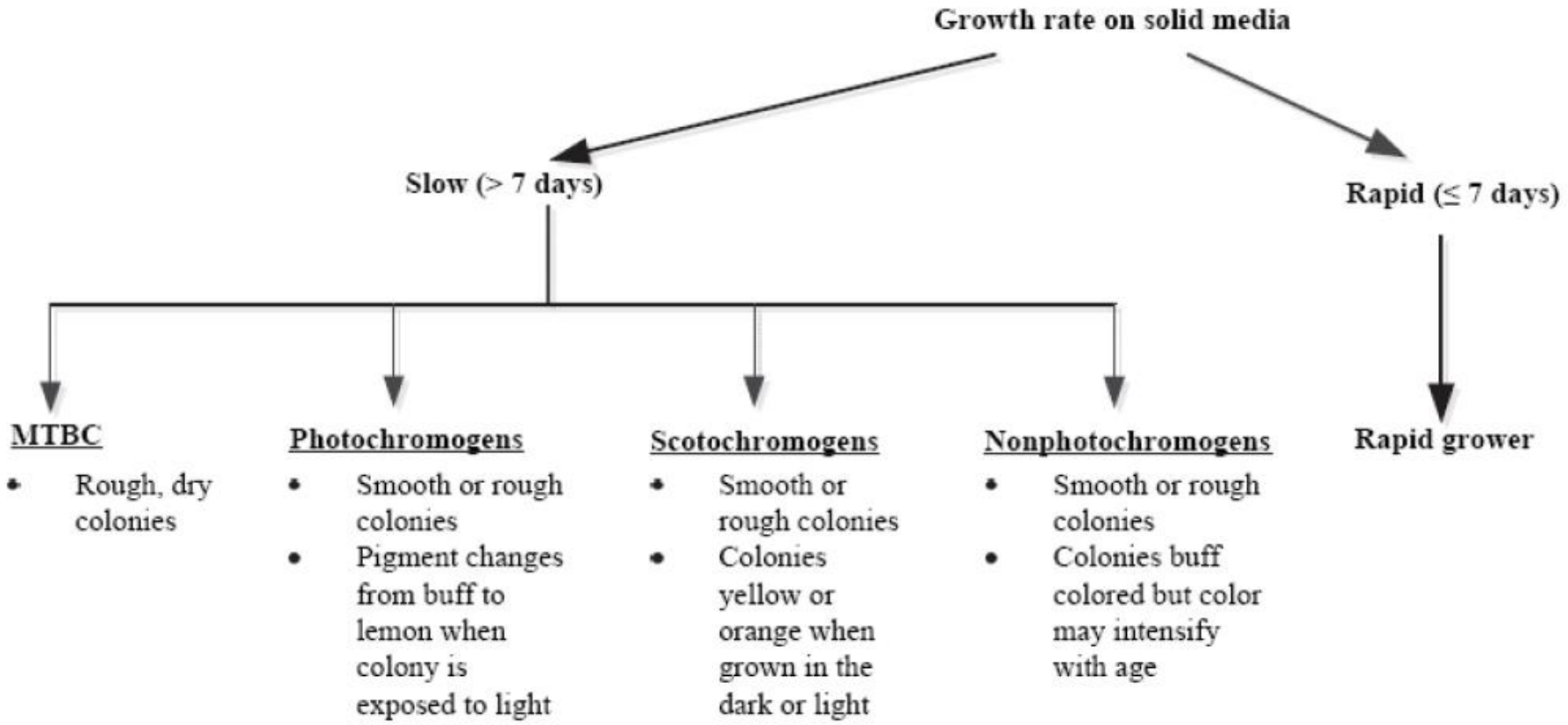
Mycobacterial identification



- **Colony morphology**

(smooth, rough)

- **Growth rate**
- **Pigmentation**

CLSI-M48, 2018

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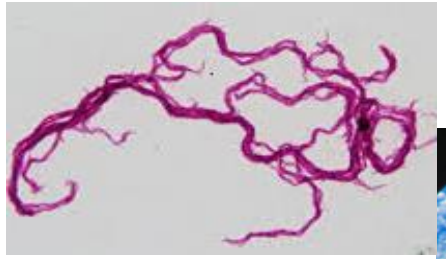
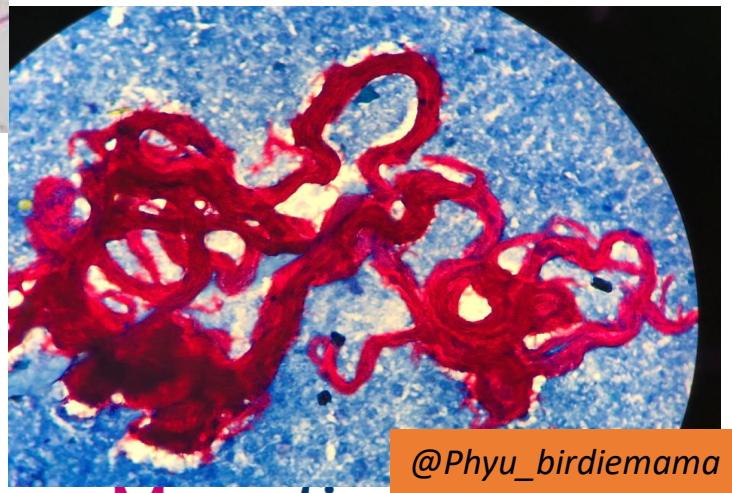
M. Tuberculosis (in absence of Accuprobe)

- Correlate with Direct MTB-PCR or smear results
- Growth rate > 7days (Liquid > Solid)
- Culture growth → "buff-colored" "rough" colonies (M. bovis = smooth colonies; ATCC attenuated strains: smooth)
- Confirmation with Kinyon/Ziehl-Neelsen stain: "cording" most cases
- ID: MALDI-ToF
  - *species level identification for M. bovis (intrinsic Pyrazinamide (PZA) resistance)





NTM species – habitats and transmission

- Most species of NTM are distributed in the **environment**
  - Water: fresh and saltwater; municipal sources
  - Soil
- Human disease is thought to be acquired from the environment
  - Inhalation, traumatic implantation, or ingestion of contaminated food/water
- Development of disease caused by SGM is dependent on host risk factors:
  - Age (older adults), body weight, chronic lung disease (CF, bronchiectasis, COPD), chest structure alterations, and environmental exposure
- There is no evidence of human-to-human transmission
  - The only animal-to-human transmission is seen with *M. marinum* (fish and fish tanks)

Clinically significant NTM's

Runyon Group	Characteristics	Species
I- Photochromogen	Pigmented production ONLY when exposed to UV/grown in light	M. kansasii M. marinum
II- Scotochromogen	Pigment production regardless of growing in dark or in light	M. gordonae M. scrofulaceum
III-Nonchromogen	Not pigmented when grown in the dark or in light	MAC M. asiaticum M. genavense M. haemophilum M. malmoense M. simiae M. szulgai M. terrae M. ulcerans M. xenopi
IV- Rapid grower	Growth on solid media in ≤7 days	M. fortuitum C M. chelonae M. abscessus M. smegmatis M. mucogenicum

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Temperature preferences – slow growing NTM

Colder

- *M. **c**helonae* (28°C)
- *M. **h**aemophilum* (30°C)
  - Also requires supplementation
- *M. **u**lcerans* (32°C)
- *M. **m**arinum* (30°C)
- *M. **m**almoense* (30°C)

CHUMM

Warmer

- *M. xenopi* (42°C)
- M. thermoresistible (42°C)
  - It's in the name ☺
- M. kansasii (some strains)

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Modified from Dr. Bekah Martin

M. kansasii

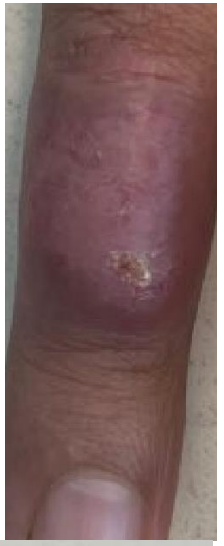
- Also causes skin and soft tissue infections, cervical lymphadenopathy, tenosynovitis, pericarditis, and rarely disseminated disease
- Closely related to non-photochromogen *M. gastri*
  - Older version of MALDI database will slash call them. Exposure to light required to differentiate them
- Photochromogen

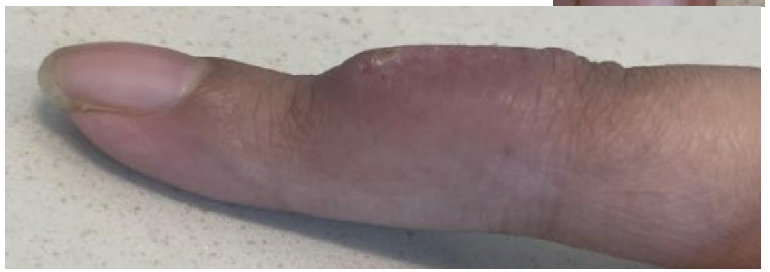
Case

- A 30-yo (13-weeks gestation) pregnant female - history of latent TB treated with 4 months of rifampin presented with 4 months of a progressively painful & ulcerated mass of her left first proximal finger joint.
- South-east Asian native; immigrated to NY 10+ years ago.

finger lesion (with vesicles)

- Punch biopsy - inflamed granulation tissue suggestive of infection
- PAS, GMS stain - Neg;
- Positive Quantiferon back in 4 months ago (when diagnosed as LTBI)
- Bacti & Fungal culture, AFB smear & culture
    - Initial Stains and smears (AFB, bacti) were all negative





Any Differentials?

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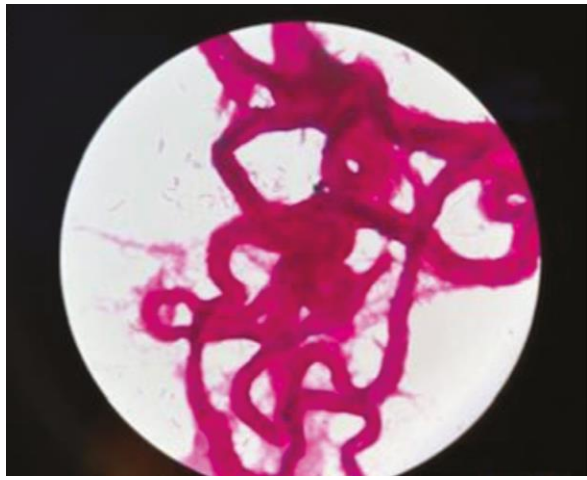
Photo credit: Dr. Molgan

Case continued



- PCR: Leishmania, Bartonella, and NTM (from skin biopsy)
- PCR Positive for *Mycobacterium marinum*
- Subsequently, a couple weeks later, AFB culture grew and Sequencing was performed & ID'd as
  - *M. marinum*
  - History revealed – cleaning of fish for cooking for family

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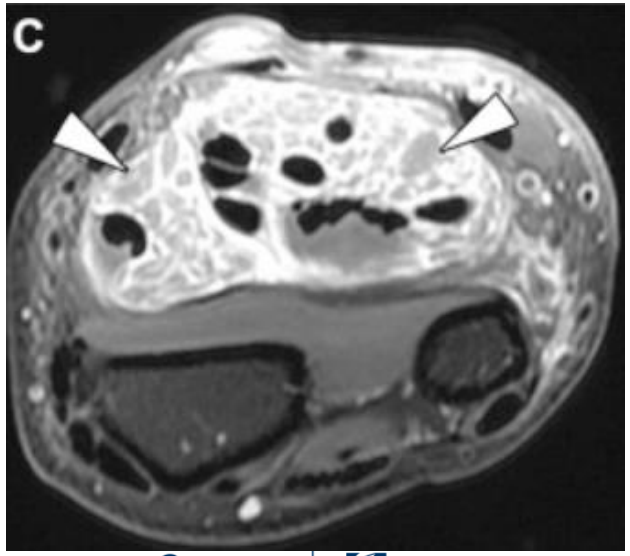
M. marinum

Yellow pigmented colonies under light
Staropoli 2008

- Grows optimally at **28 – 32ºC**
  - Other NTM that require lower temp for growth: *M. haemophilum*, *M. ulcerans*
  - Typically cause skin/soft issue/bone/joint infections, therefore, **AFB wound cultures are incubated at 5 – 10% CO_2 at 35 – 37ºC and 30 – 32ºC**

- Runyon Group I: photochromogenic, grows in 8 – 12 days

- Clinical features:
  - Single, painless papule on extremity 2 – 3 weeks after inoculation (may also cause OM, tenosynovitis, septic arthritis)
  - Causative agent of “swimming pool” or “fish tank” granulomas due to injury in fresh water, salt water, fish-tanks

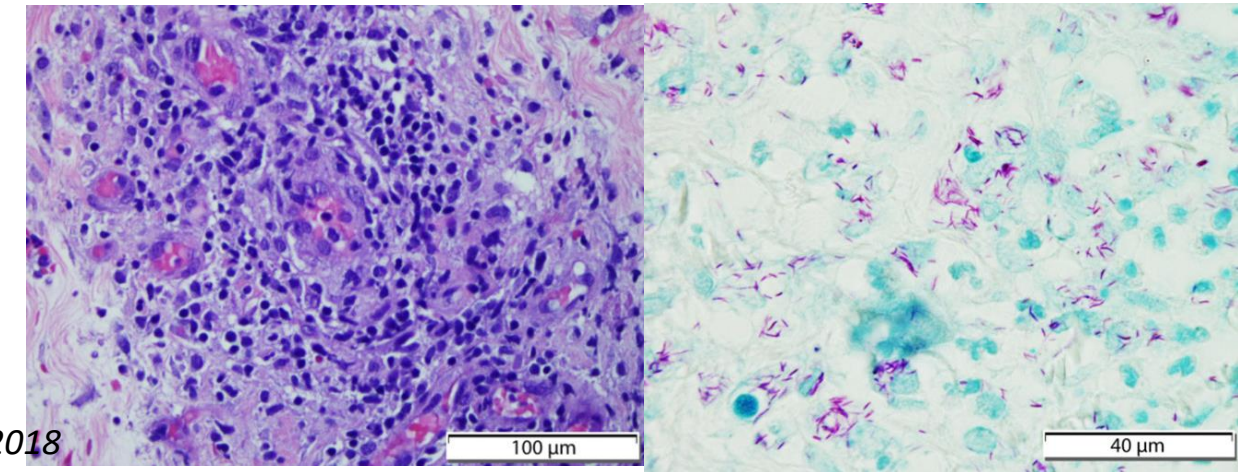


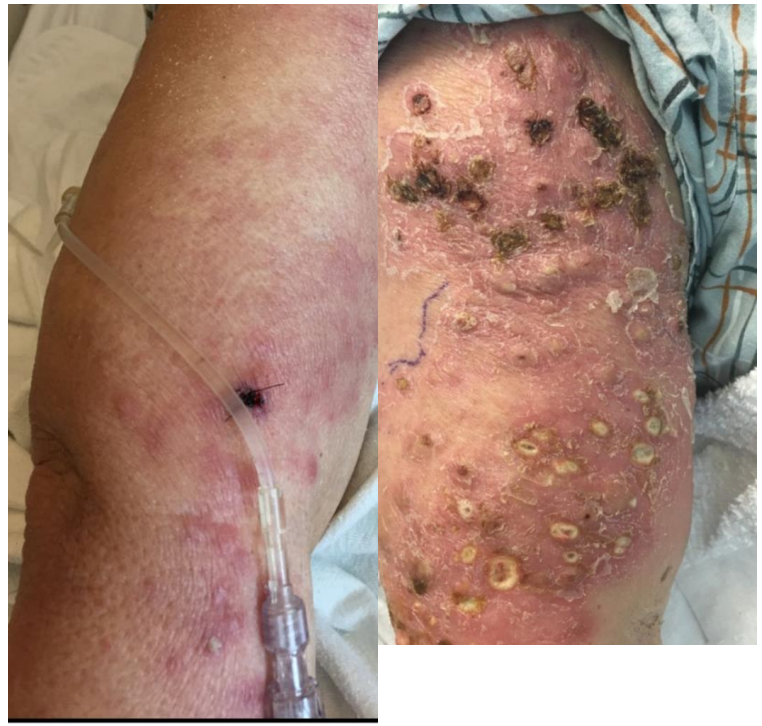
Presence of ”rice bodies” within mass lesion seen on MRI wrist (Jiang et al 2015)

Case in our backyard

- A 65 yo female from Thailand, kidney transplant recipient 7 years ago due to ESRD, was hospitalized for fevers and rash.
- Patient diagnosed with polymorphic Epstei-Barr virus + post transplant Lymphoproliferative d/o
- On exam: ill-appearing, mild fever, right eye – nodular conjunctival lesion; Skin – erythematous papules. Central pustules on forehead, face, neck, arms, knees, lower back.
- TST positive; HIV neg; Quantiferon Neg;
- Skin biopsies: Path stainin
- Path Staining: numerous A stain: rare AFB
- Cx – 2 weeks NG; worsened condition → sequencing of tissue block
- 44 days later: Chocolate agar at 30C grown → AFB

M. hemophilum





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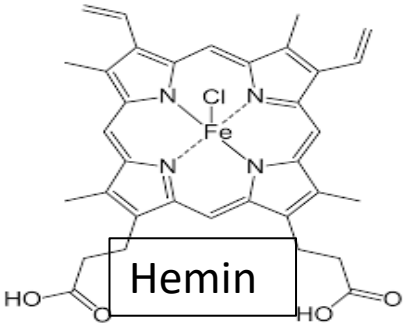
Jacobs et al. JCM. Brief case. 2018

M. hemophilum

Iron



Hemin

- **Fastidious**
  - **Media supplementation with iron-containing compounds (ferric ammonium citrate, or hemin – X factor)**
  - **"satellite growth" – growth around paper strip ("X-factor strip") impregnated w/ Hemin on 7H10/11 agar – presumptive ID – cost effective**
  - **Requires incubation at lower temperatures (28-30^{o}C)**
  - **Adding Choc agar plate for specimens (extremities)**

- Commonly presents as multiples skin nodules on the extremities
- Lymphadenitis or pulmonary nodules in otherwise healthy pediatric patients
- Can give false positive Tuberculin skin test (TST) due to cross-reactivity

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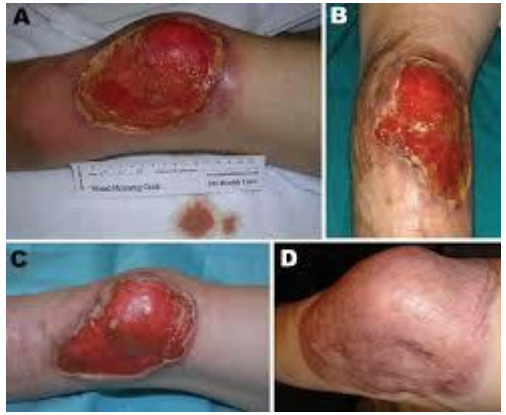
Jacobs et al. JCM. Brief case. 2018; modified –Dr. Bekah Martin

M. ulcerans

- Third most common cause of mycobacterial disease worldwide [1: MTBC, 2: *M. leprae*]
- “Buruli ulcer” in Africa (after county in Uganda)
- “Bairnsdale ulcer” in Australia
- Transmitted through traumatic implantation in an extremity. Begins as a painless nodule/lump, ulcerating overtime eventually becoming widespread ulcerative necrosis or osteomyelitis
- Tissue damage caused by mycolactone production (toxin)
- **Fastidious: grows best at cool temps (28-30C) and extended incubation (up to 3 months)**



M. xenopi

- First isolated from skin lesions on the African toad (*Xenopus laevis*)
- Causes chronic respiratory disease, skin and soft tissue infections, septic arthritis, and disseminated disease (in immunocompromised hosts)
- Prefers **higher temperatures (45C) – "thermophile"**
- Frequently isolated from hot water systems and tanks
- Growth – is still supported by MGIT (Liquid media)
- Identification - MALDI-ToF (latest database) accurate ID

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Modified from Dr. Bekah Martin

M. gordonae

- "Tap-water bacillus"
  - Found in soil and water sources
- One of the most commonly recovered mycobacteria in the laboratory
- Generally considered a contaminant/non-pathogenic
  - Repeated isolation in the context of appropriate clinical correlation is required to consider it a pathogen
  - Susceptibility is not routinely performed or needed
- Scotochromogen

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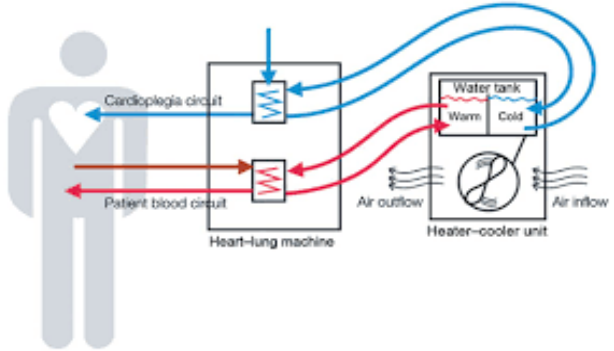
Koneman's color atlas; modified –Dr. Bekah Martin

Mycobacterium avium Complex (MAC)

- Most commonly isolated NTM from respiratory infections
- Predominant species for human infection include:
  - *Mycobacterium avium*
  - *Mycobacterium intracellulare* *(primary respi pathogen; less common in disseminated infections)
  - *Mycobacterium chimaera*
- A few subspecies designation
- *M. chimaera:* Heater-cooler related HAI (open heart surgery)



FDA U.S. FOOD & DRUG ADMINISTRATION

FDA's Ongoing Evaluation and Continued Monitoring of Reports of Nontuberculous Mycobacteria Infections Associated with Water-Based Heater-Cooler Devices

EMERGING INFECTIOUS DISEASES

Invasive Nontuberculous Mycobacterial Infections among Cardiothoracic Surgical Patients Exposed to Heater–Cooler Devices[1]

Meghan M. Lyman,[✉] Cheri Grigg, Cara Bicking Kinsey, M. Shannon Keckler, Heather Moulton-Meissner, Emily Cooper, Minn M. Soe, Judith Noble-Wang, Allison Longenberger, Shane R. Walker, Jeffrey R. Miller, Joseph F. Perz, and Kiran M. Perkins

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MAC – laboratory identification

- Slow-growing NTM
  - 10-35 days to grow on solid media
- Smooth (or rough), transparent or opaque, light tan
- 30-37^{o}C (*M. chimaera* 25-37)
- Non-photochromogens
- Niacin and nitrate reduction negative

MALDI Identification: ***M. chimaera* reported out by MALDI as MAC**

(downstream impact?? – Rx management?) -

Forbes et al. Practical guideModified Dr. Bekah Martin;

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Clinically significant NTM's

Etiological agents of disseminated disease

- MAC (typically in HIV positive individuals)
- *M. genavense* (HIV positive and severely immunocompromised)
- *M. kansasii* (rare)
- *M. malmoense* (disseminated disease and septic arthritis are rare)
- *M. xenopi* (disseminated disease and septic arthritis in immunocompromised)

Etiological agents of cutaneous diseases

- *M. haemophilum* – skin nodules on the extremities in immunocompromised hosts (AIDS patients)
- *M. kansasii* – skin and soft tissue infections
- *M. leprae*
- *M. marinum* – exposure to fish or fresh/saltwater
- *M. ulcerans* – Buruli ulcer
- *M. xenopi* – skin and soft tissue infections

Etiological agents of pulmonary diseases

- MAC (most common presentation of this org)
- *M. abscessus* group
- *M. kansasii* (most common presentation of this)
- *M. malmoense* (In adults with known pulmonary disease)
- *M. simiae*
- *M. szulgai*
- *M. xenopi*

Case

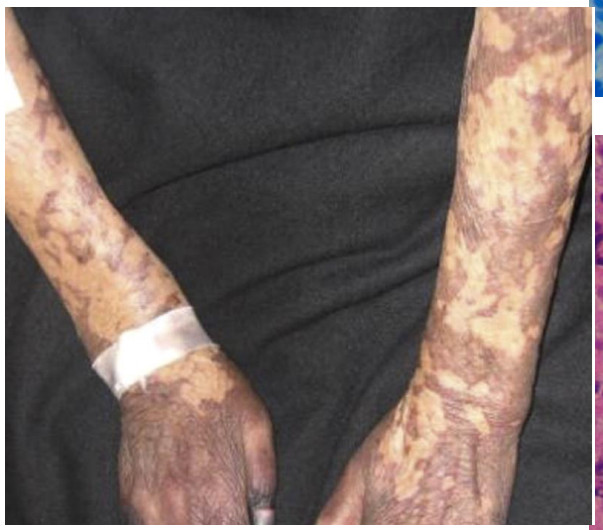
- An 86 yo female originally from NE Mexico presented with diffuse skin lesions on her extremities
- No known apparent risk factors; generally healthy
- Skin biopsy performed; biopsy staining (fig)
- Biopsy sent for AFB culture and smear - smear neg; culture – NG.

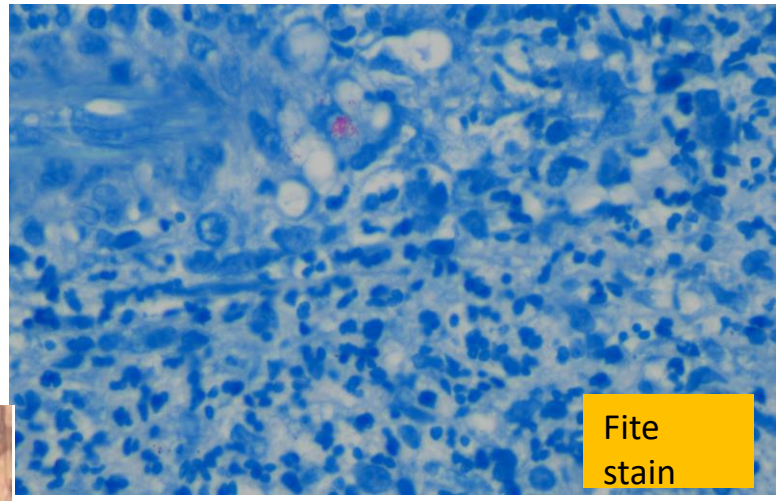
What is the likely pathogen?

A) *M. ulcerans*

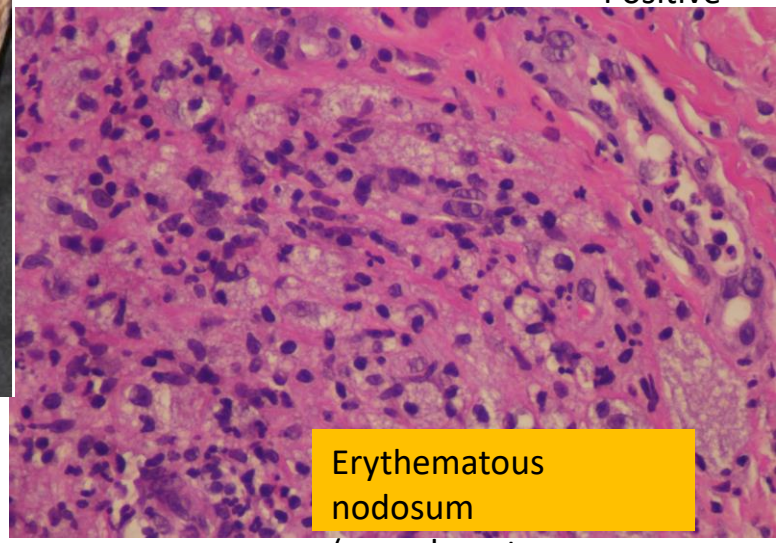
B) *Non-TB, Non-NTM,* some kind of mycobacterium?

C) Culture set up missed lower temperature incubation – likely one of those NTM that requires lower temperature incubation





Fite stain Positive



Erythematous nodosum (granulomatous lesion)

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JCM: Case report: DOI: https://doi.org/10.1128/jcm.05634-11

Microscopic images credit to Dr. Bijal Amin, M.D

Case -- continued

What is the likely pathogen?

A) M. ulcerans

***B) Non-TB, Non-NTM,* some kind of mycobacterium?**

C) Culture set up missed lower temperature incubation – likely one of those NTM that requires lower temperature incubation

Mycobacterium leprae

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M. leprae

- Cause of leprosy (Hansen's disease) - chronic granulomatous disease affecting skin & peripheral nervous system and mucous membrane

- Person-to-person spread: inhalation of droplets
- Pauci vs. multi-bacillary disease (pauci = milder form)
- Sanatoria – management of leprosy

- California, Florida, Hawaii, Louisiana, NY and Texas ➔ 70% of total reported cases
- Unculturable (diagnosis mostly based on clinical S&S)
- Incubate in nine-banded Armadillos (mouse footpads in research labs)

Rapid growers

I. M. fortuitum complex

Frequently encountered environmental organism (opportunistic pathogen)

II. M. chelonae complex*:* more frequently assoc with SSTI than pulmonary;

III. M. abscessus group *(*Second most common NTM pulmonary infection followed by MAC)

- *M. abscessus subsp. abscessus :* ~ 80% of *M. abscessus* infection
- *M. abscessus subsp. Massiliense ~ 15% of* clinical isolates; recovery rate has increased lately
- *M. abscessus subsp bolletii* – uncommon in US

- Possess *erm* gene – resistance gene (macrolide): ~ 85% *M. abscessus* (most *M. abscessus subsp. Massiliense* – non-functional erm gene)

** Sub-species level identification ** →

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Summary of Identification approaches and methods

- Growth characteristics
  - Growth rate : > or < 7 days
  - Temperature
  - Colony morphology
  - Pigment production

  Conventional biochemical methods
- HPLC
- Line Probe Assays
- GenProbe accuprobe
- MALDI-ToF
- DNA sequencing

Initial smear +
Direct NAAT results

COLLEGE of AMERICAN PATHOLOGISTS

MIC.32140	Rapid Method	Phase I
	A rapid method (nucleic acid probes, chromatography, the NAP test, matrix-assisted laser description ionization time-of-flight (MALDI-TOF) mass spectrometry, nucleic acid amplification, or sequencing) is employed for identification of mycobacterial isolates.	

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Bio-break Before a cool case?

Case – TB or not TB?

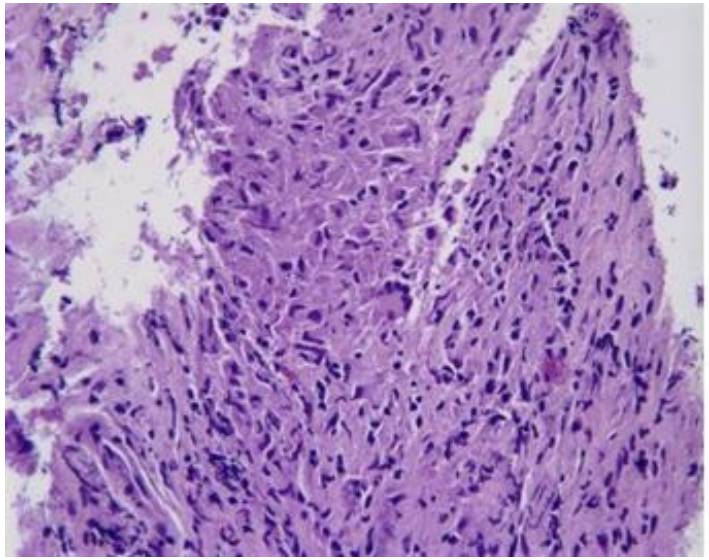
- 65 yo male with Parkinson's disease, HTN, CKD, & hypothyroidism → ED (Hypotension, hypoglycemia, unresponsiveness)
- Admitting dx: Adrenal insufficiency
- PMH: Remote **TB** treated 20-30 yrs ago; incarcerated; ***Histoplasma capsulatum*** (dimorph mold)

	Day 2	Day 6
Histoplasma Galactomannan Ag Interp, Urn		Not Detected
Histoplasma Galactomannan Ag Quant, Urine		Not Detected
Mitogen minus Nil	8.773	
Nil	0.084	
QFT Gold Plus Result	Positive (A)	
TB1 minus Nil	9.938	
TB2 minus Nil	9.844	

	Day 1	Day 7	Day 12
SYPH IgG/IgM			Non-reactive
HIV 1/2 Ag-Ab with Reflex	Negative		
HIV Semi-quantitative	0.06		

TB or not TB - continued

- Rt adrenal CT image guided FNA & Core biopsy
- Surgical path:
  - Adrenal gland tissue with necrotizing granulomatous inflammation; No malignant cells identified.
  - GMS neg; AFB stain neg;
- Micro: MTB-PCR of adrenal biopsy (off-line: a part of on-the-go validation sample) **negative**;
- Additional tests: To rule out Histoplasma, Coccidiodes, CMV (usually in HIV)



Additional Micro cultures ordered

- AFB – smear and culture of the biopsy: Neg and NGTD
- Fungal Culture – NGTD

(1 week old cx)

- Repeat PCR requested, repeat sampling;

utmb Health

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Any clues? TB or not TB??

- Following week (2.5 weeks) – culture (biopsy) grew AFB → *M. tuberculosis*

So, why is MTB-PCR neg and AFB smear neg?

utmb Health

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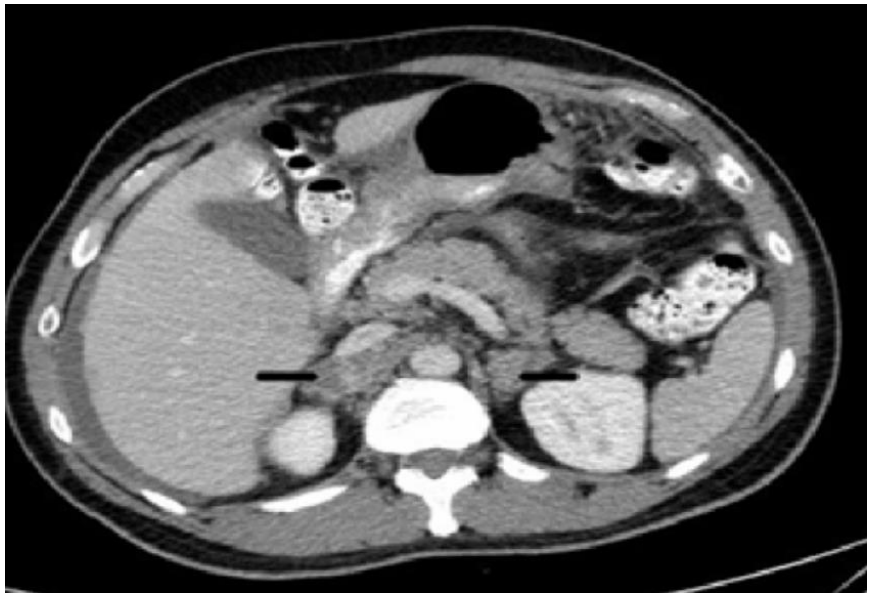
So, why is MTB-PCR neg and AFB smear neg?

- Adrenal biopsy sample for MTB-PCR testing - Off-label test (validation in process at the time)
- Sampling – biopsy tissue → paucibacillary in nature

Diagnosis: Adrenal Tuberculosis



utmb Health

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Extra-pulmonary Tuberculosis

- TB disease – burden worldwide
- Socio-economic differences and health disparities
- Asian immigrants (36%) and Hispanic/Latino (30%)
- TB in residents of correctional facilities

- Not airborne or person-to-person not transmissible (unlike airborne respiratory TB)
- About 15-20% - EP dissemination

Extra-pulmonary TB prevalence in the U.S

Year	Total Extrapulmonary Cases[1]	Total Extrapulmonary Sites[2]	Site of Disease															
			Pleural		Lymphatic		Bone or Joint		Genitourinary		Meningeal		Peritoneal		Laryngeal		Other	
			No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
2010	2,412	2,516	393	(15.6)	1,012	(40.2)	270	(10.7)	115	(4.6)	138	(5.5)	140	(5.6)	2	(0.1)	446	(17.7)
2011	2,170	2,291	391	(17.1)	864	(37.7)	251	(11.0)	117	(5.1)	132	(5.8)	122	(5.3)	3	(0.1)	411	(17.9)
2012	2,077	2,186	365	(16.7)	843	(38.6)	226	(10.3)	110	(5.0)	103	(4.7)	126	(5.8)	4	(0.2)	409	(18.7)
2013	1,970	2,080	355	(17.1)	748	(36.0)	222	(10.7)	103	(5.0)	109	(5.2)	121	(5.8)	4	(0.2)	418	(20.1)
2014	1,925	2,032	333	(16.4)	778	(38.3)	210	(10.3)	97	(4.8)	98	(4.8)	117	(5.8)	4	(0.2)	395	(19.4)
2015	1,905	2,008	343	(17.1)	720	(35.9)	194	(9.7)	90	(4.5)	92	(4.6)	126	(6.3)	3	(0.1)	440	(21.9)
2016	1,869	1,973	323	(16.4)	702	(35.6)	193	(9.8)	92	(4.7)	85	(4.3)	121	(6.1)	2	(0.1)	455	(23.1)
2017	1,887	2,015	320	(15.9)	764	(37.9)	181	(9.0)	84	(4.2)	87	(4.3)	119	(5.9)	0	(0.0)	460	(22.8)
2018	1,828	1,936	327	(16.9)	715	(36.9)	185	(9.6)	92	(4.8)	74	(3.8)	113	(5.8)	3	(0.2)	427	(22.1)

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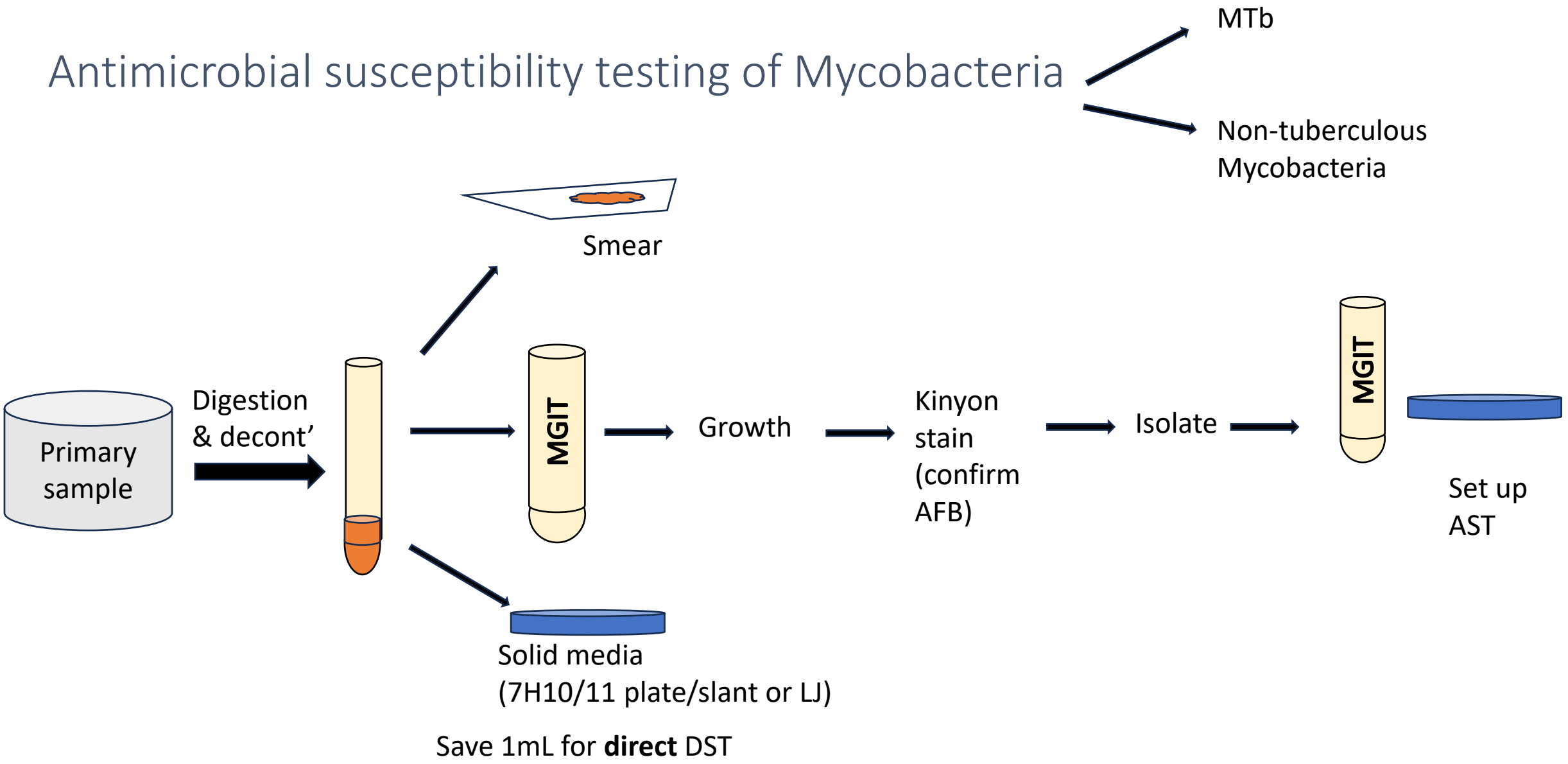
CDC

AST

Antimicrobial susceptibility testing of Mycobacteria



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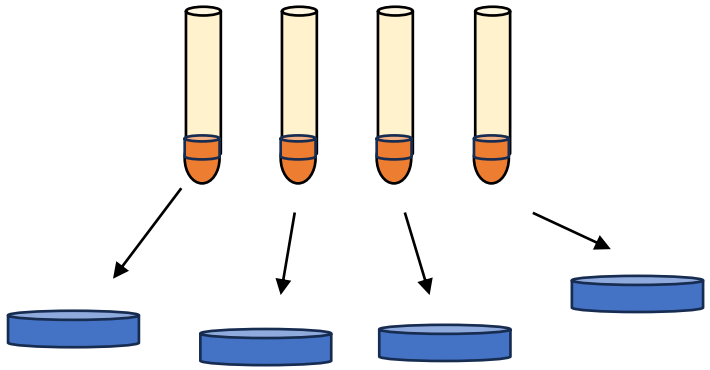
AST testing methodologies

Agar proportion

Different dilution of standardized innoculum



+/- drug containing
Middle brook 7H10/ 7H11

Incubate → Count colonies

- Definition of resistance: >1% of growth in the drug-containing medium compared to drug free medium

- Cost effective, Capture hetero-resistance colonies
- Time consuming & labor intensive
- Used to be gold standard but not anymore

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Other AST testing methods

Commercial broth AST System

- Liquid broth micro dilution – eg. MGIT or Versa Trek
- Must use drug concentration recommended by regulatory agencies (CLSI/FDA)

Antituberculous Agent	System and Concentration, µg/mL		
	Fluorescence-based Detection System	Pressure-based Detection System	Agar Proportion Middlebrook 7H10 Equivalent
Isoniazid	0.1	0.1	0.2
Isoniazid	0.4	0.4	1.0
Rifampin	1.0	1.0	1.0
Ethambutol hydrochloride	5.0	5.0	5.0
Ethambutol hydrochloride	7.5†	8.0	10.0
Pyrazinamide	100	300	–‡
Streptomycin	1.0	–†	2.0
Streptomycin	4.0	–†	10.0

CLSI M24, 2018. Appendix C

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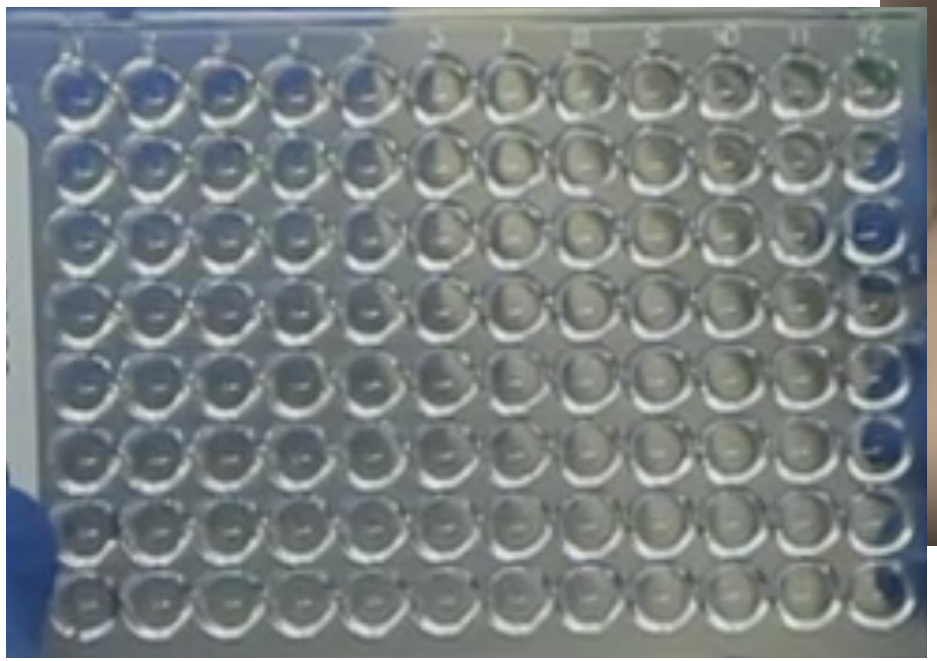
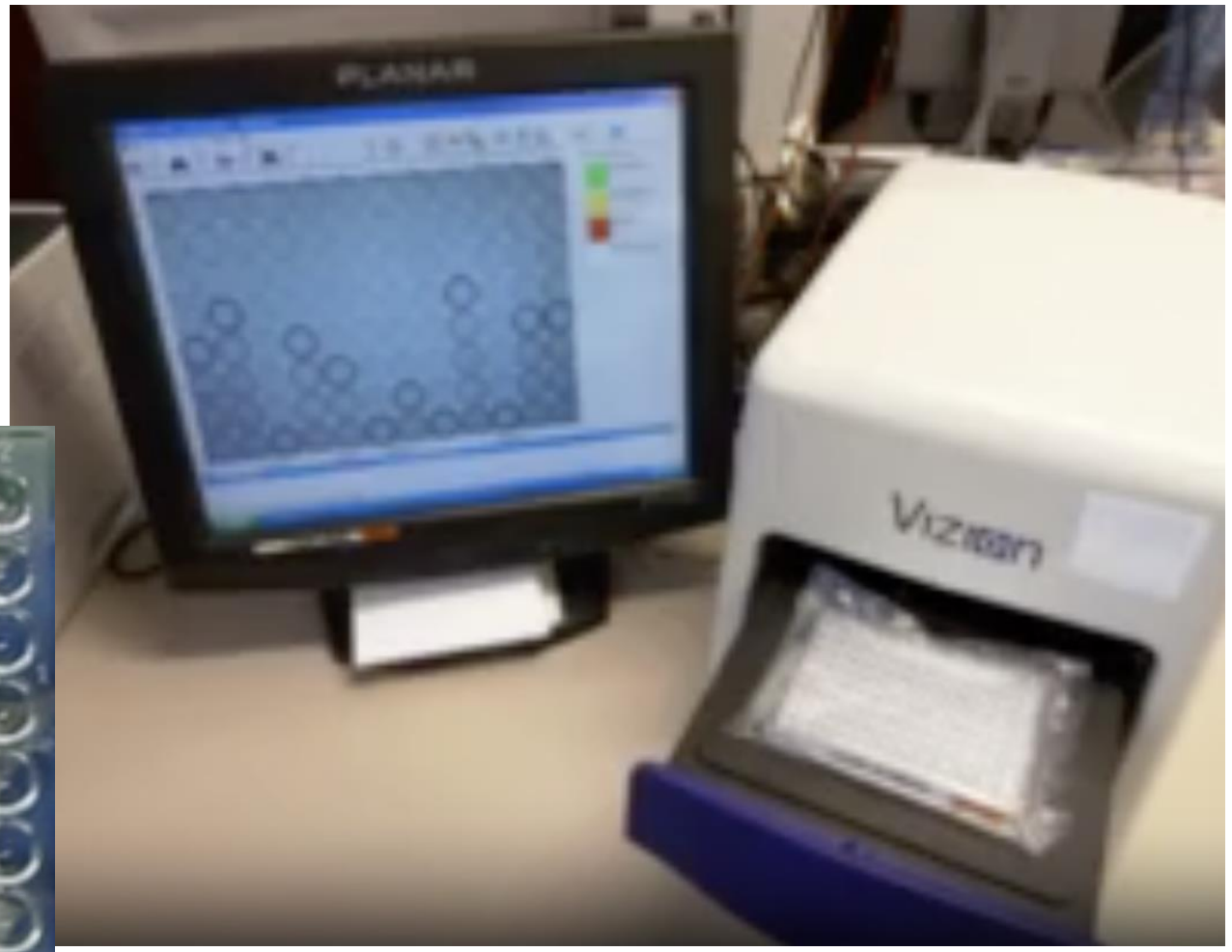
Other AST testing methods

Broth microdilution panels

- Must seal the plates after drug/bug inoculation
- Manual read vs. automated read





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Hall et al. 2011. J Vis Exp. PMID: 21730945

AST – Tuberculous vs. non-tuberculous mycobacteria

MTB susceptibility testing

Four first-line drugs need to be tested (RIPE)

- **R**ifampin (Rif)
- **I**soniazid (INH)
- **P**yrazinamide (PZA)
- **E**thambutol (EMB)

- First isolate from each patient; repeat every 2-3 months (if subsequent cx continues to grow)
- Suscept testing **should be** reported **within 17 days (CLSI)** of reporting identification; CAP – 28 days of specimen receipt
- If initial testing reveals resistance, repeat testing to confirm resistance (rule out NTM /contaminant)
- Resistance can be confirmed by molecular methods – Rif & INH

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CLSI M 24, 2018; CAP

Antimicrobial susceptibility testing of NTM

When should NTM susceptibility be performed?

- Clinically significant isolates (Eg., Blood, sterile body fluids, tissues, skin and soft tissue infections)

- Criteria for Respiratory isolates
  - At a minimum TWO NTM culture positive sputa or ONE Bronch wash or lavage
  - A transbronchial or lung biopsy with histopathologic support and a positive culture

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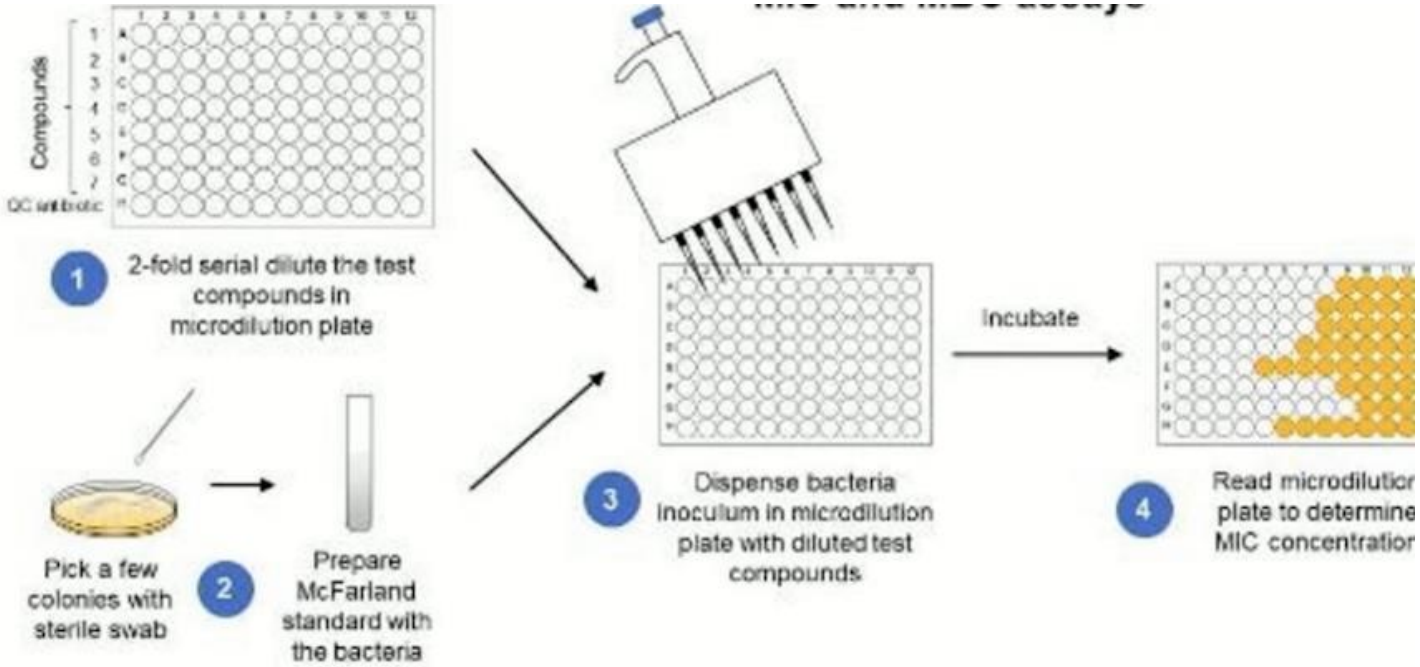
Brown-Elliot.Antimycobacterial susceptibility testing of Nontuberculous Mycobacteria. JCM 2019

Antimicrobial susceptibility testing of NTM

- Broth microdilution – “ gold standard” recommendation by CLSI
- 96-well microtiter plate
- McFarland 0.5; two-fold dilution series; incubation at 30 & 35C. 3, 7 D
- Both MIC and interpretation should be reported



RGM: NO E-tests!!!

Modified from Barbara Brown-Elliot; CLSI 2018

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Rapid growers' susceptibility (resistance) profile

	Inducible resistance to macrolides? (separate from constitutive resistance)	
M. fortuitum	Yes, *erm* gene	
M. chelonae	No*	
M. abscessus	subsp. *abscessus*	Yes, *erm* gene
	subsp. *massiliense*	No, *erm* gene is non-functional due to large deletion
	subsp. *bolletii*	Yes, *erm* gene

- 2023: newly recognized inducible *erm* gene in *M. cheloane* and two other Mycobacterium species (iranicum & obtuense).
- CLSI recommendation : 3-14 days of incubation (reporting 3 D vs. extended incubation to 14 days for final report)

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A few notable points about NTM susceptibility testing & Overall Quality assessment

- Truly specialized testing area – required rigorous training (for all types of NTM)
- No commercial proficiency testing program (so far) for NTM AST

Indicator	Recommended Target	Numerator	Denominator
AFB smear	Within 24 hours of specimen receipt in the laboratory	Number of AFB smear results reported within target TAT	Total number of AFB smear results reported
NAAT	Within 48 hours of specimen collection	Number of NAAT results reported within target TAT	Total number of NAAT results reported
NAAT use[175]	Positive NAAT reported on 77% of culture-confirmed TB patients	Number of patients with positive NAAT results	Total number of culture-confirmed TB cases
MTBC ID from culture	Within 21 days of specimen receipt in the laboratory	Number of MTBC results reported within target TAT	Total number of MTBC IDs reported
TB first-line AST results	Within 17 days of MTBC ID from culture	Number of AST results reported within target TAT	Total number of AST results reported

CLSI M48, 2018

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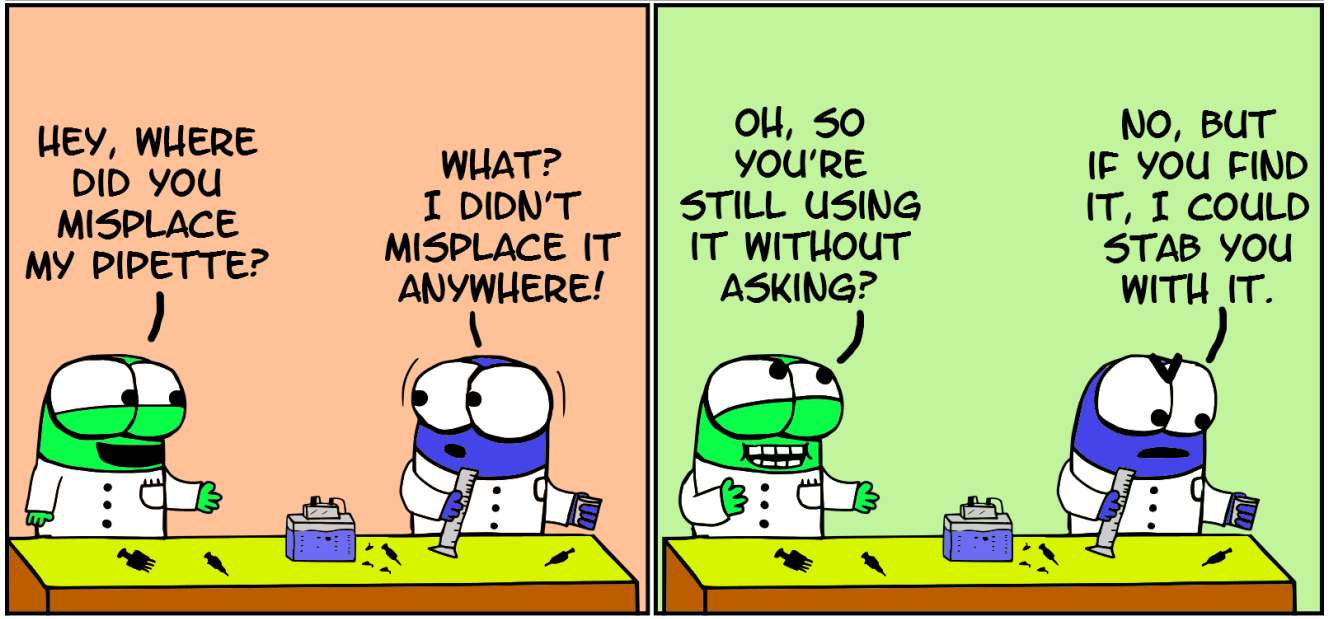
THE UPTURNED MICROSCOPE PRESENTS

Logical Fallacies in the Lab

TODAY: **LOADED QUESTION**

ASKING A QUESTION THAT CONTAINS A PRESUMPTION, SO THAT IT IS IMPOSSIBLE TO ANSWER WITHOUT ACCEPTING IT (AND APPEARING GUILTY).



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Ref

- Somoskövi A, Hotaling JE, Fitzgerald M, O'Donnell D, Parsons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy. Chest. 2001 Jul;120(1):250-7. doi: 10.1378/chest.120.1.250. PMID: 11451846; PMCID: PMC2925666.

What is the most immediate clinical application (benefits) of direct AFB smear results?

Choose all that applies:

A) To differentiate between TB vs. non-TB infections

B) To remove infection isolation (quarantine) with three-smear negatives

C) To manage treatment in known positive TB case

D) To confirm culture growth

What clinical (Infection prevention & control) use is beneficial from direct AFB smear results?

Choose all that applies:

A) To differentiate between TB vs. non-TB infections

B) To remove infection isolation (quarantine) with three-smear negatives

C) To manage treatment in known positive TB case

D) To confirm culture growth