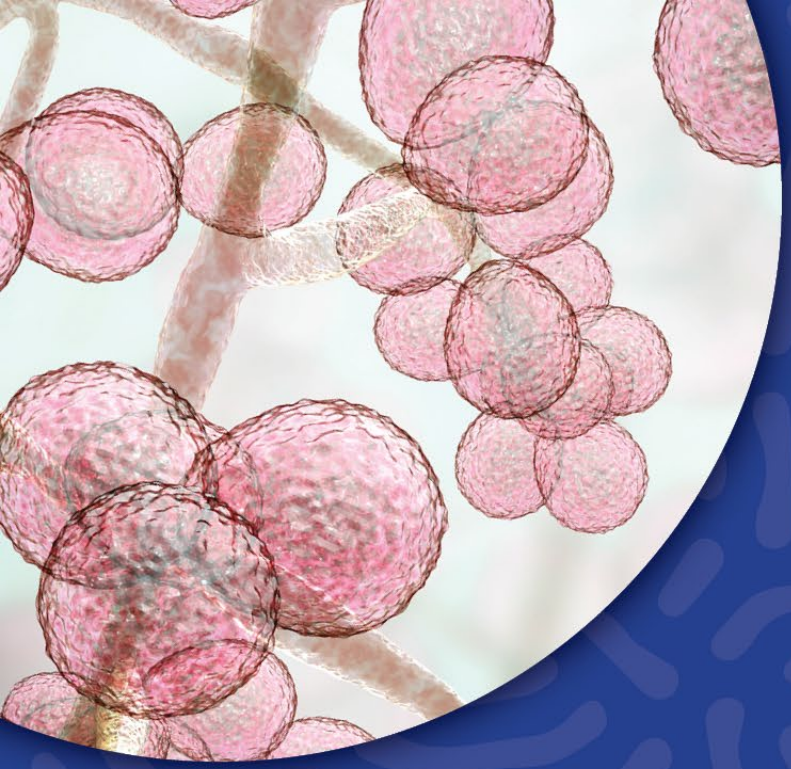
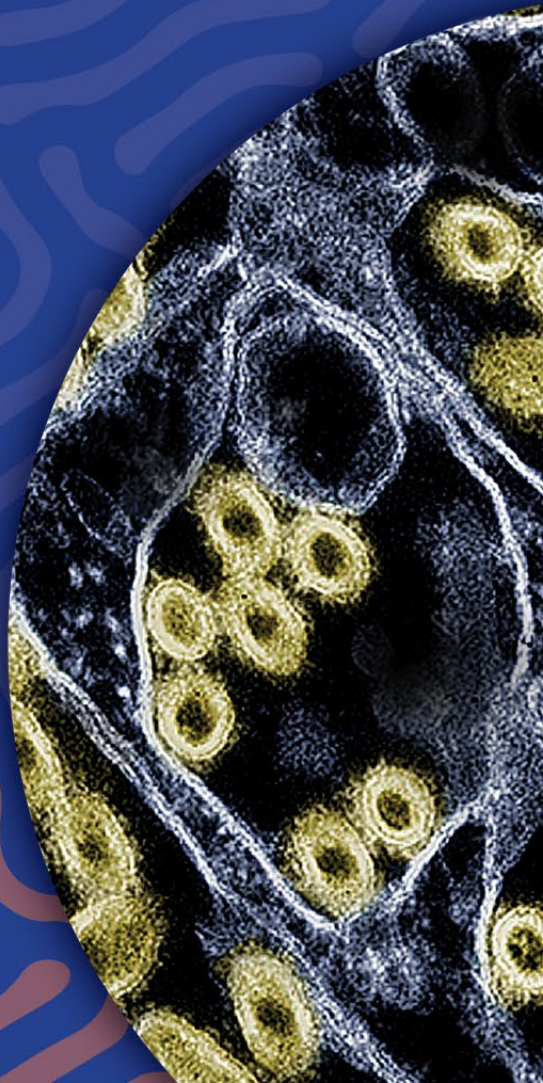


ID LAB CON

APHL

2025

#IDLabCon



Workup of Clinical Samples for NTM in a Clinical Laboratory

#IDLabCon

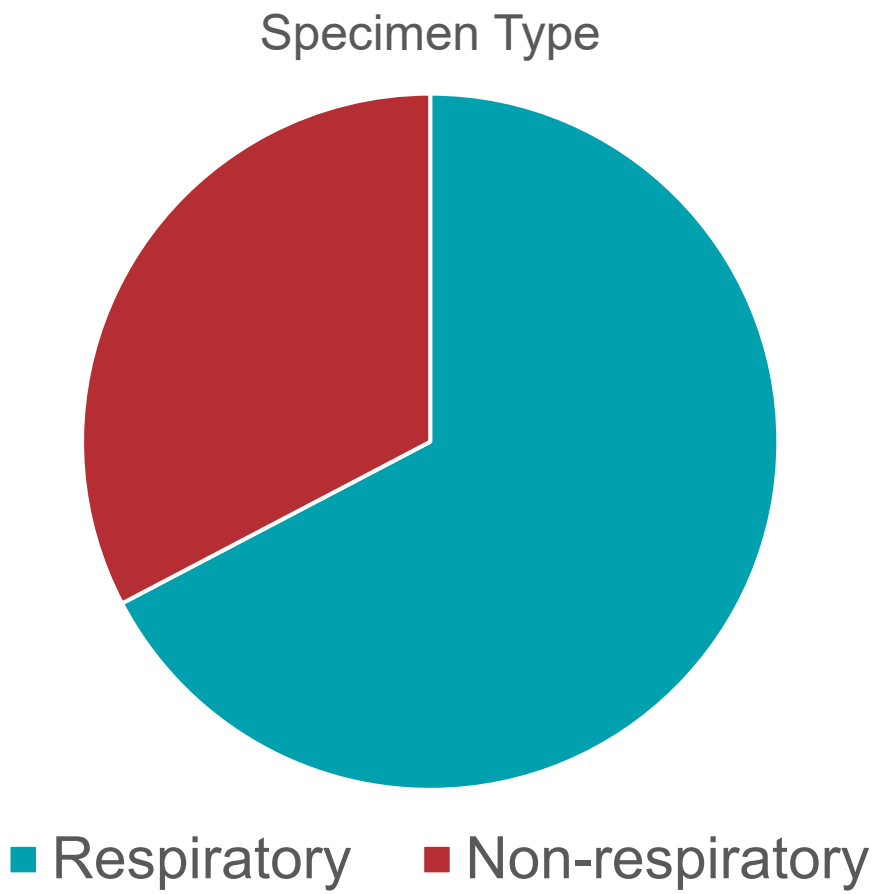
Susan Butler-Wu, PhD, D(ABMM), FIDSA
Director, Clinical Microbiology
Los Angeles General Medical Center
Associate Professor, Clinical Pathology
Keck School of Medicine of USC

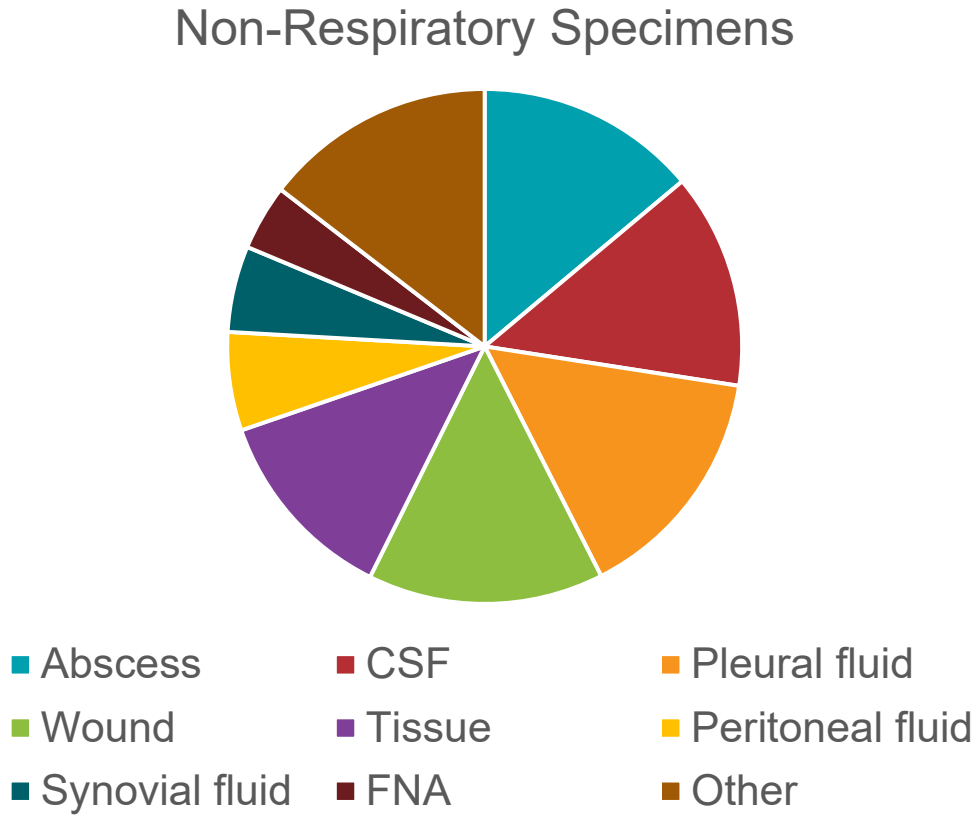
LA General Medical Center

- Largest full service public hospital on West Coast (600-beds)
- Large, busy ED – Level 1 trauma center
- Large immigrant and underserved patient population
- Diagnose 2% of active tuberculosis cases in the United States
- Limited LDTs (n = 2), highly dependent on reference lab testing
- Limited resources

AFB cultures at LA General

- ~ 4,500 AFB cultures per year





Specimen management & processing

- Send-out urine & stool specimens for AFB culture
- NALC-NaOH decontamination performed on respiratory specimens, non-sterile body fluids (e.g. abdominal, peritoneal, etc.)
- Induced & expectorated sputum for AFB smear & culture x3
  - **two of the specimens for in-house TB PCR (one direct, one on concentrate)**
- Sterile body fluids: no decontamination performed
  - CSF, synovial, pleural, pericardial, amniotic, ascites fluids
- Smear: Rhodamine-Auramine
  - performed once daily, soon to perform x2/daily

Culture practices

Media	Specimen Type	Inoculation	Incubation	Temp.	Length of incubation
Middlebrook 7H11	All sample types	3 drops	6-10% CO_2	35-37°C	7 weeks*
Chocolate agar	Skin, bone & joint specimens (incld. joint/synovial fluids)	3 drops	6-10% CO_2	35-37°C	7 weeks*
MGIT tube	All samples *other* than blood	0.5 mL	MGIT 960: Non CO_2	35-37°C	7 weeks*

*10 week incubation if AFB seen on smear

- MycoLytic/F in process of being validated for blood culture, currently sent out to reference lab

Work-up of AFB-positive specimens in a clinical laboratory

Accuprobe for MTBC, MAC, *M. kansasii*, *M. gordonae*

- could be performed on both isolates and AFB+ broth
- probe negative? Report as NTM and send to reference lab for identification
- probes discontinued in 2022

MALDI-TOF Mass Spectrometry

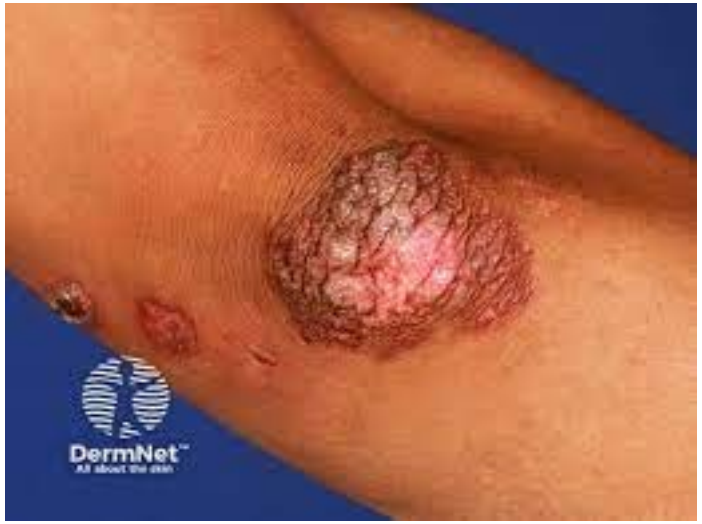
- **could be performed on both isolates and AFB+ broth**
- bioMerieux system: extraction kit and database FDA-cleared for mycobacteria
- Bruker system: RUO for mycobacteria; 2023: MBT HT Mycobacteria Module & MBT Mycobacteria Kit which are both RUO

ID LAB CON
APHL 2025

#IDLabCon

Is this the case for MALDI & positive broths?

- 31 yo M presents to dermatology for evaluation of chronic right lower leg lesions
  - believes that he may have had a bite or stepped on something while bathing in the river in Oaxaca as a young child
- Patient is originally from Mexico, living in LA for the last year
- Started on his thigh, but lesions have now spread to his right foot
- Lesions have increased in size over time, becoming more painful
- Denies any other rashes, fevers/chills, shortness of breath or any other symptoms
- No known contacts with similar lesions
- Years of burning/cauterizing/freezing the lesions in Mexico
- Also has had multiple biopsies in Mexico, at one point was diagnosed with sporotrichosis per the patient, but he is unsure if he was treated
  - biopsy at an OSH in LA was unrevealing
- Dermatologist's initial DDx included: chromoblastomycosis & other fungal infections, mycetoma, leishmaniasis, chronic granulomatous disease, squamous cell carcinoma



(Not patient's actual lesion)

- Biopsy showed acute/chronic inflammation with fibroplasia and follicular cystic changes
- Infectious stains were negative:
  - PAS, Fite, T. *pallidium*, HHV8
- Culture grew 4+ *Staphylococcus aureus*, 2+ *Fusobacterium necrophorum*
  - **AFB culture discontinued due to bacterial overgrowth**
  - Fungal culture was negative
- Patient was prescribed Clindamycin for 10 days
- On follow-up, no improvement in lesions. Patient underwent an additional biopsy of his thigh lesion

What happened next…

- Patient was re-biopsied
- Bacterial culture grew coagulase-negative *Staphylococcus* from broth only
- AFB culture: No AFB seen on smear
- MGIT positive for growth of AFB at 3 weeks
- MALDI performed on the positive MGIT tube – **no identification**

And now we wait…

Either wait for colonies to grow and/or send AFB+ broth to reference lab for ID

☹

#IDLabCon

Is “no ID” from broth a common problem???

Identification of AFB from broth culture

- Performance of MALDI for AFB growing in liquid broth has been variable (69%-99%)

Study	Medium	Time from +ve	Database	Study Set	Results
Buchan et al	Versa TREK	24-48h	Bruker v1.0	88 isolates	80.2%, SV≥2.0
Kehrmann et al	MGIT	N/S	VITEK MS (Saramis 4.12)	160 isolates, 24 spp of NTM	76.9% id
Chien et al	MGIT	N/S	N/S	92 sequential NTM	47.8%
Park et al	MGIT & Versa TREK	1-2 weeks	Bruker v1.0	206 isolates	83.5% with SV >1.7, 55.8% ≥2.0
Quinlan et al	MMBacT Alert 3D	24-48h	Bruker Biotyper 3.1	151 isolates	96.4% of MTC vs. 52.3% of NTM
van Eck et al	MGIT	24-48h	Bruker v2.0	54 prospective specimens	22% ID'd from liquid vs. 91% for colonies (>1.7); 13% ≥2.0

More recently...

TABLE 2 Vitek MS v3.0 library performance for identifying *Mycobacterium* spp. in liquid medium

Reference ID[a]	No. (%) of isolates with indicated Vitek MS v3.0 ID results according to identification category (confidence value)			
	Excellent (≥99.9%)	Good (60.0–<99.9%)	No ID (<60.0%)	Mis-ID
NTM slow growers				
M. avium complex				
M. avium (*n* = 75)	51 (68)	9 (12)	15 (20)	
M. intracellulare (*n* = 271)	203 (67.2)	21 (7.7)	68 (25.1)	
MAC-X[c] (*n* = 36) (includes but may not discriminate among *M. colombiense**, *M. marseillense**, *M. timonense**, *M. chimaera**, and *M. yongonense**)	11 (30.6)		25 (69.4)	
*M. angelicum** (*n* = 2)			2 (100)	
M. asiaticum (*n* = 2)			2 (100)	
M. gordonae (*n* = 17)	11 (64.7)	1 (5.9)	5 (29.4)	
*M. interjectum** (*n* = 1)			1 (100)	
M. kansasii (*n* = 111)	80 (72.1)	8 (7.2)	23 (20.7)	
M. lentiflavum (*n* = 3)	3 (100)			
M. mantenii (*n* = 1)			1 (100)	
*M. parascrofulaceum** (*n* = 6)			1 (16.7)	5 (83.3)
*M. seoulense** (*n* = 1)			1 (100)	
*M. shigaense** (*n* = 1)			1 (100)	
M. szulgai (*n* = 3)	3 (100)			
M. triplex (*n* = 2)			2 (100)	
M. xenopi (*n* = 7)	2 (28.6)		5 (71.4)	
NTM rapid growers				
M. abscessus (*n* = 284)	244 (85.9)	9 (3.6)	31 (10.9)	
M. chelonae (*n* = 5)	4 (80)		1 (20)	
M. fortuitum group (*n* = 23) (includes but does not discriminate between *M. porcinum*, *M. peregrinum*, and *M. septicum**)	18 (78.3)[b]		5 (21.7)	
*M. monacense** (*n* = 2)			2 (100)	
M. neoaurum (*n* = 1)	1 (100)			
*M. wolinskyi** (*n* = 1)			1 (100)	
Total (*n* = 855)	610 (71.4)	48 (5.6)	192 (22.4)	5 (0.6)

- 106 NTM in liquid broth (tested at 12-72h) – 90.6% correctly identified with VITEK MS[2]

#IDLabCon

[1]Luo L et al. *Microbiol. Spectrum*. 2022. 02018-22
[2]Rodriguez-Temporal D et al. *Micrbiol. Biotechnol*. 2023. 16(4): 778-783

Is this a biomass issue?

Table 3 Score comparison for two NTM isolates after extended incubation

	M. abscessus		*M. gordonae*	
	Automatic spectral acquisition	Manual spectral acquisition	Automatic spectral acquisition	Manual spectral acquisition
Day 1	NPF	N/A	NPF	N/A
Day 3	NPF	1.725	NPF	1.756
Day 5	NPF	1.903	NPF	1.949
Day 7	NPF	1.920	1.929	2.024
Day 14	NPF	1.939	2.029	2.037

N/A, not applicable; NPF, no peaks found; NTM, non-tuberculous mycobacteria.

Quinlan P, *et al. J Clin Pathol* 2015;**68**:229–235. doi:10.1136/jclinpath-2014-202374

- Identification rate for MALDI-ToF-MS performed on AFB+ broth was only 60% at LA General

ID LAB CON
APHL 2025

#IDLabCon

Time for something completely different?

Rapid differentiation of MTBC from NTM

Validation of Xpert MTB/Rif assay for positive MGIT broths

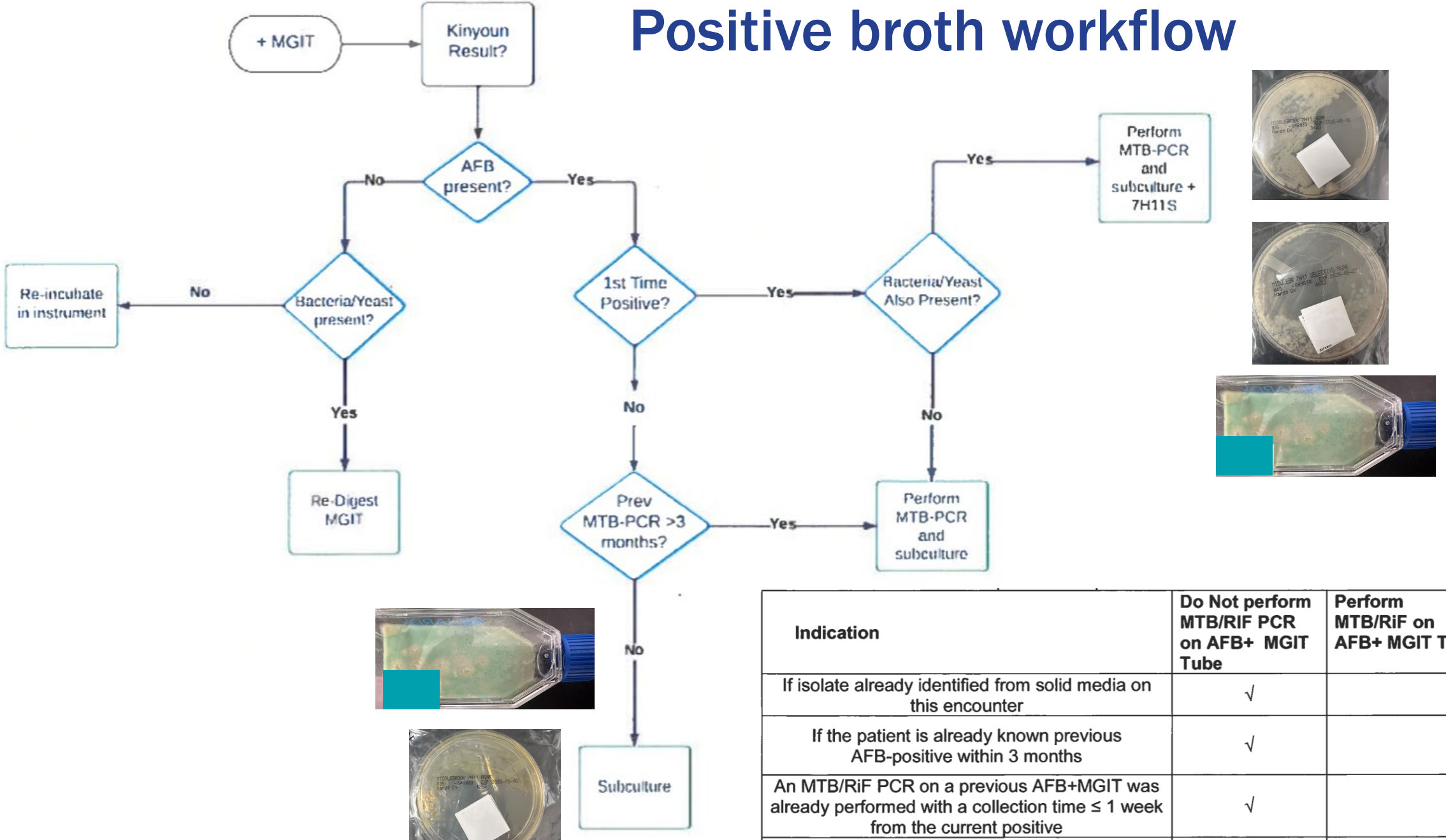
TABLE 2 Identification of MTBC and RIF resistance for clinical isolates and spiked MGIT tubes (all SR ratios)

Category (no. of isolates)	Species/strain (no. of isolates)	GeneXpert MTB/RIF result (no. of isolates/total no. of isolates)
Clinical isolate testing (100)	MTBC, RIF-S (10)	*M. tuberculosis* detected, RIF not detected (10/10)
	NTM (90)	*M. tuberculosis* not detected (90/90)
	M. abscessus (14)	*M. tuberculosis* not detected (14/14)
	M. chimaera (1)	*M. tuberculosis* not detected (1/1)
	M. fortuitum (8)	*M. tuberculosis* not detected (8/8)
	M. gordonae (9)	*M. tuberculosis* not detected (9/9)
	M. kansasii (2)	*M. tuberculosis* not detected (2/2)
	M. avium-M. intracellulare (54)	*M. tuberculosis* not detected (54/54)
	M. marinum (1)	*M. tuberculosis* not detected (1/1)
	M. peregrinum (1)	*M. tuberculosis* not detected (1/1)
Spiked MGIT cultures (112)	MTBC, ATCC strain H37Rv (10)	*M. tuberculosis* detected, RIF not detected (10/10)
	MTBC, RIF-R[a] (24)	*M. tuberculosis* detected, RIF detected (24/24)
	MTBC plus bacteria (20)	
	MTBC (RIF-S) plus *S. aureus* (5)	*M. tuberculosis* detected, RIF not detected (5/5)
	MTBC (RIF-S) plus *E. coli* (3)	*M. tuberculosis* detected, RIF not detected (3/3)
	MTBC (RIF-R) plus *S. aureus* (8)	*M. tuberculosis* detected, RIF detected (8/8)
	MTBC (RIF-R) plus *E. coli* (4)	*M. tuberculosis* detected, RIF detected (4/4)
	Incubated MGIT tubes (34)	
	ATCC strain H37Rv (11)	*M. tuberculosis* detected, RIF not detected (11/11)
	MTBC, RIF-R[a] (11)	*M. tuberculosis* detected, RIF detected (11/11)
	ATCC strain *M. fortuitum* (4)	*M. tuberculosis* not detected (4/4)
	ATCC strain *M. kansasii* (4)	*M. tuberculosis* not detected (4/4)
	ATCC strain *M. avium* (4)	*M. tuberculosis* not detected (4/4)
	MTBC limit of detection (24)	
	MTBC only, 10^6 CFU/mL (4)	*M. tuberculosis* detected (4/4)
	MTBC only, 10^3 CFU/mL (4)	*M. tuberculosis* detected (4/4)
	MTBC only, 10^1 CFU/mL (4)	*M. tuberculosis* not detected (4/4)
	MTBC plus *M. avium-M. intracellulare*, 10^6 CFU/mL (4)	*M. tuberculosis* detected (4/4)
	MTBC plus *M. avium-M. intracellulare*, 10^3 CFU/mL (4)	*M. tuberculosis* detected (4/4)
	MTBC plus *M. avium-M. intracellulare*, 10^1 CFU/mL (4)	*M. tuberculosis* not detected (4/4)

[a]Rifampin-resistant mutations include D516Y (isolate 48), H526Y (isolate 58), and S531L (isolates C1, MP1, and 67).

- Categorical Agreement = 100% for identification of MTBC and detection of rifampin resistance
- No false-positive results or cross-reactivity for 45 spiked MGIT tubes (n=14 distinct NTM species)
- MTBC PCR negative, AFB+ MGIT reported as presumptive NTM at LA General

Positive broth workflow



Indication	Do Not perform MTB/RIF PCR on AFB+ MGIT Tube	Perform MTB/RiF on AFB+ MGIT Tube
If isolate already identified from solid media on this encounter	√	
If the patient is already known previous AFB-positive within 3 months	√	
An MTB/RiF PCR on a previous AFB+MGIT was already performed with a collection time ≤ 1 week from the current positive	√	
On first time AFB+MGIT tube		√

#IDLabCon

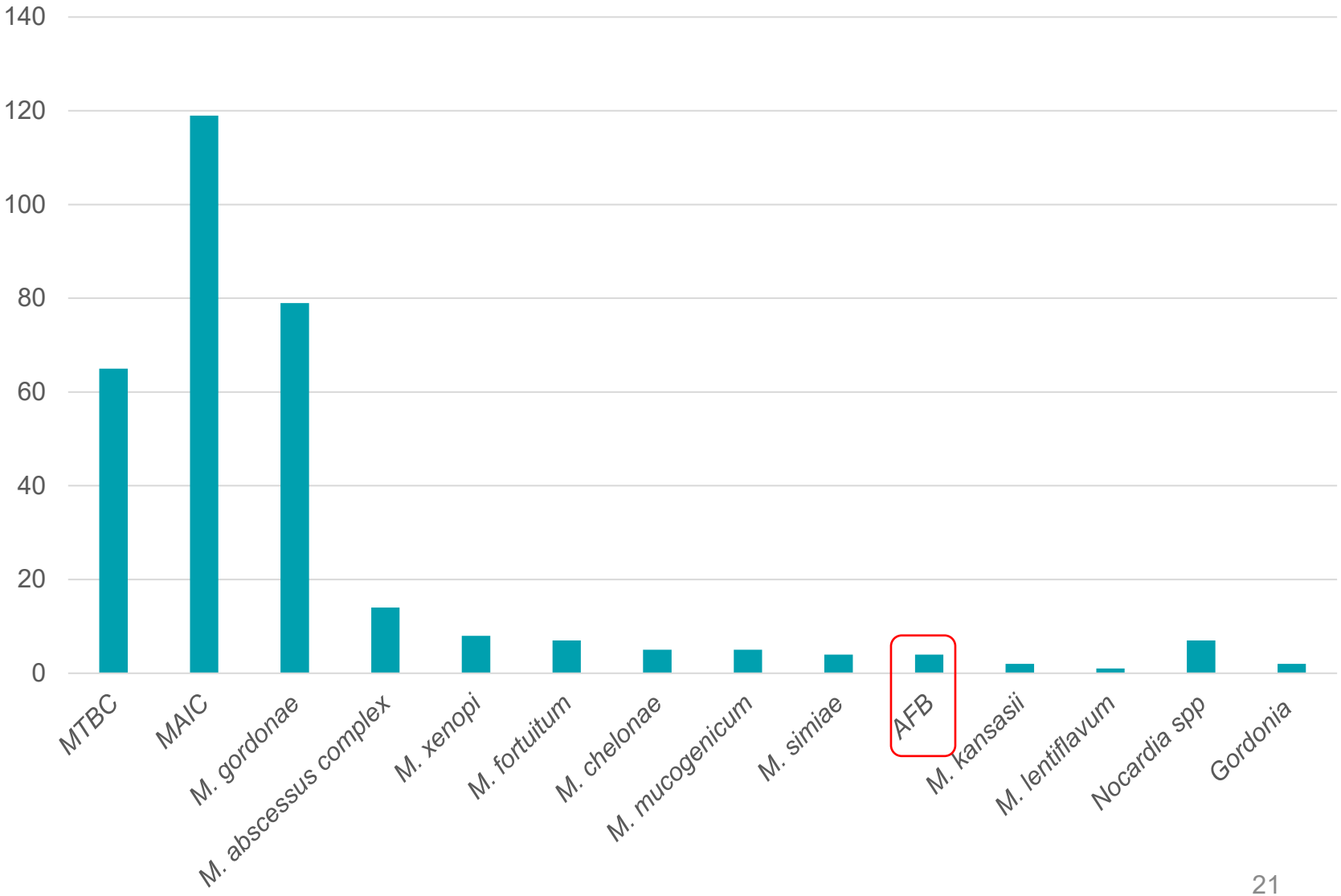
Now back to the patient...

Case conclusion

- Performed MTB/RIF assay on the patient's AFB-positive MGIT broth
  - validated as an LDT in our lab*
- Positive for ***Mycobacterium tuberculosis* complex**
- Patient was started on TB therapy
- All respiratory specimens were negative x3 & his chest X-ray was completely normal
- Lesions resolving at his most recent appointment! ☺

***Shout out to Dr. Thao Truong, Josue Kattan, & Rosario DeLosAngeles for their MGIT TB PCR validation work!**

AFB Epidemiology at LA General (2024, Q3/Q4)



- AFB positivity = 20.2%
- 20.2% of AFB isolated in lab were *M. tuberculosis* complex
- 98.8% of positive cultures are identified with current testing approach

Diagnostic gaps at LA General (& beyond)

- No in-house susceptibility testing for MTBC
- No in-house susceptibility testing for NTM
- NTM susceptibility testing performed upon provider request
- No ability of routine MALDI-ToF-MS to differentiate members of *M. abscessus* complex
- Molecular detection of *erm*(41) and *hsp65* genes is costly & not widely available in most reference labs
- Need a rapid molecular test for NTM identification from broth!

And we're <u>really</u> lucky to have in-house culture for AFB!

Discontinuation of NTM probes has made clinical labs reliant on MALDI-ToF-MS for identification from broth culture

Molecular identification of TB from broth culture is rapid and efficient

Many opportunities for future molecular testing on MGIT broths for NTM identification – but needs to be “sample-to-answer”

Though currently necessary, reliance on reference labs increases cost and turnaround time