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Advanced Diagnostic Laboratories

From Researcher to Patient: The Role of the Laboratory in Diagnostics and Treatment

Max Salfinger, MD
Director, Mycobacteriology & Pharmacokinetics Laboratories
salfingerm@njhealth.org

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Topics

- Introduction
- Tool Box 1 & 2
- Growth Detection
- Identification of NTM
- Antimicrobial Susceptibility Testing (AST)
- Survey 2010 & 2017

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Financial Disclosure

NONE to report

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P-NTM Species

Four integrated health care delivery systems*, 1991-2007

• ***M. avium* complex**	1,495	(80.1%)
• ***M. chelonae/abscessus***	225	(12.1%)
• *M. fortuitum*	106	(5.6%)
• *M. kansasii*	102	(5.5%)
• *M. simiae*	53	(2.8%)
• *M. xenopi*	33	(1.7%)

*KP Southern California, KP Southern Colorado, Group Health, Geisinger

AJRCCM 2010 Prevots et al.

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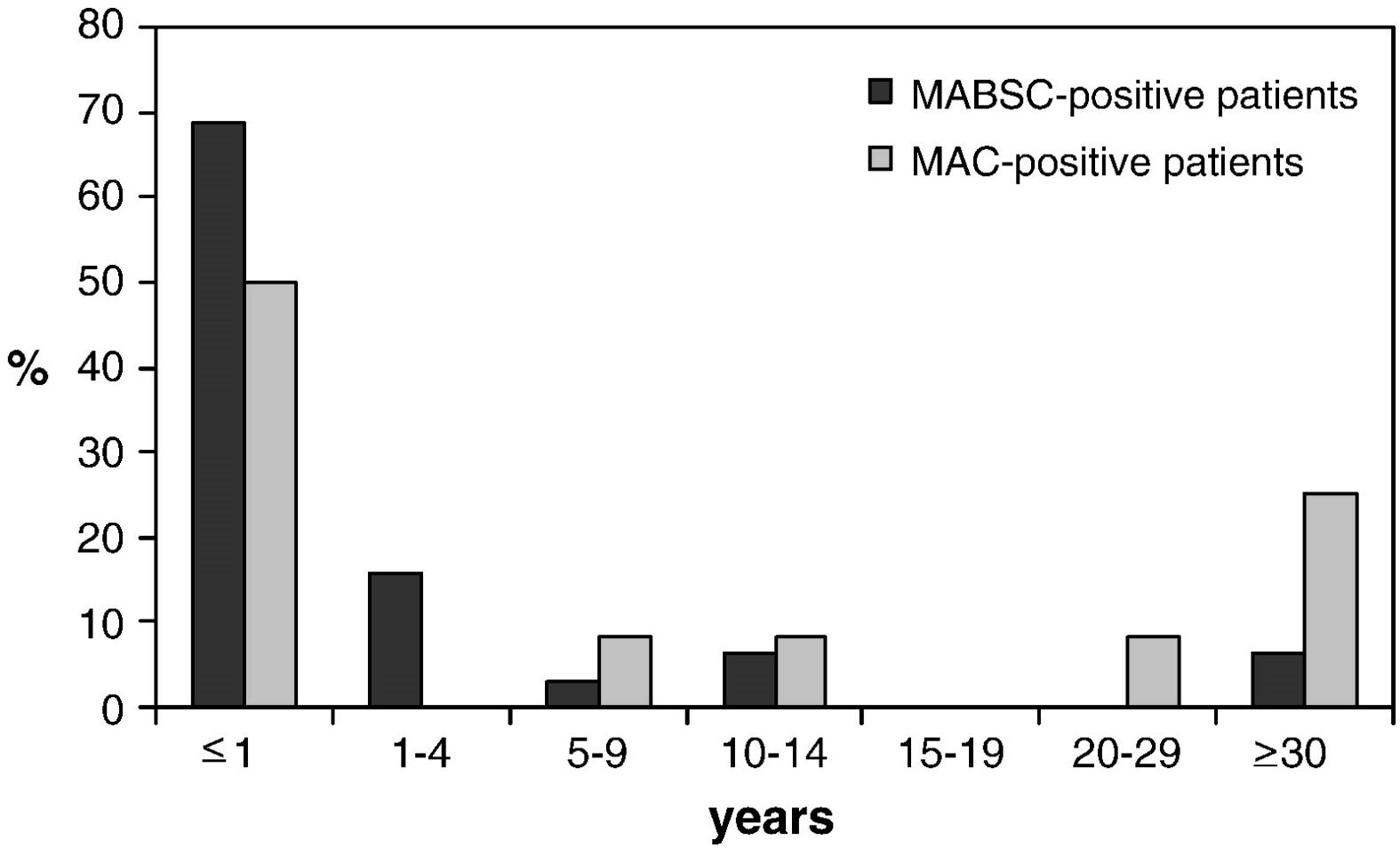
NTM in CF – Age Related



Catherinot et al. J Cyst Fibros. 2013 Jan;12(1):74-80

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Toolbox-1

- ✓ **Specimen – sputum, bronchoscopy, formalin-fixed tissue**
  - **NALC-NaOH versus Oxalic acid (CF w/history of *Pseudmonas aeruginosa*)**
  - **AFB microscopy**
  - **Solid (LJ, Middlebrook bi-plate, NTM plate) & broth-based media**
  - **NAAT-D (TB complex, NTM –mostly MAC)**
  - **NAAT-R (RIF, INH and more)**
  - **Direct AST**

Ideally, molecular testing 7 days a week

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Toolbox-2

- ✓ **AFB positive culture (broth-, solid-based media)**
  - **NAAT-D (TB complex)**
  - **NAAT-R (RIF, INH and more; NTM- macrolide & aminoglycoside)**
  - **Identification (Sequencing-*rpoB* or 16S; MALDI-TOF; Nucleic acid probe kits; Line Probes)**
  - **High Performance Liquid Chromatography (HPLC); PCR Restriction Analysis (PRA); Biochemicals**
  - **Minimal Inhibitory Concentration (MIC) (rapidly and slowly growing NTM)**
  - **Combination MIC**

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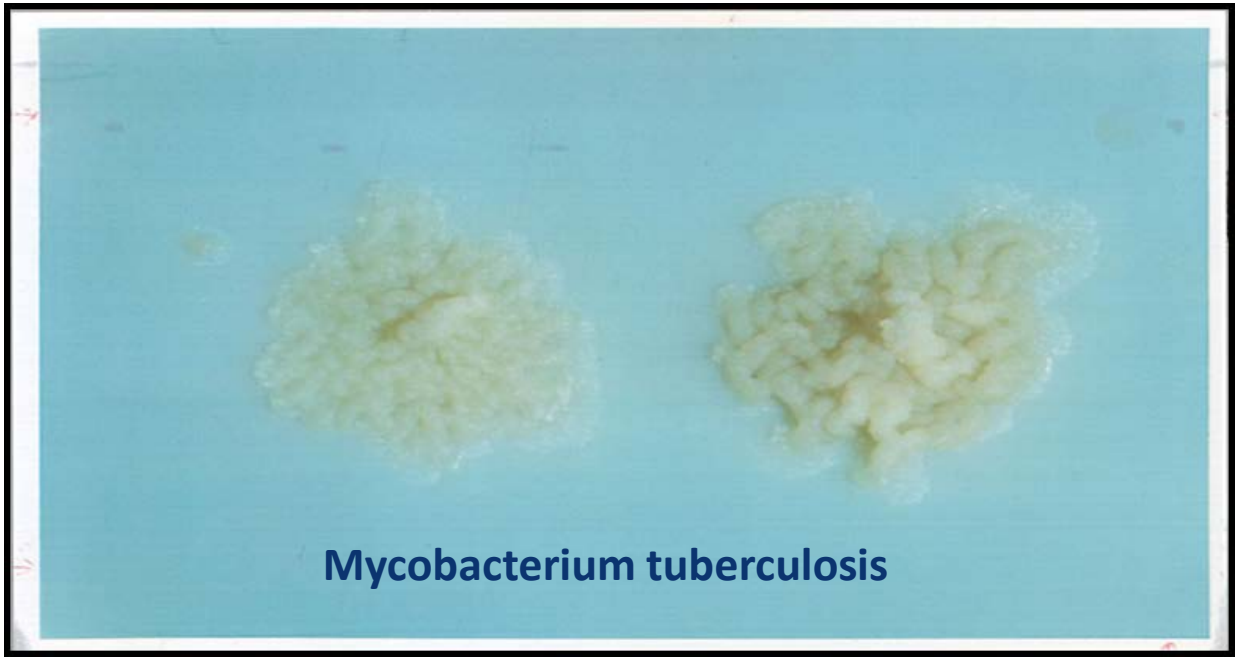
Processing Sputum

- Procedures kill all but 10-20% of the mycobacteria
- Contamination
  - 2-5% of sputum specimens on Loewenstein-Jensen medium (LJ)



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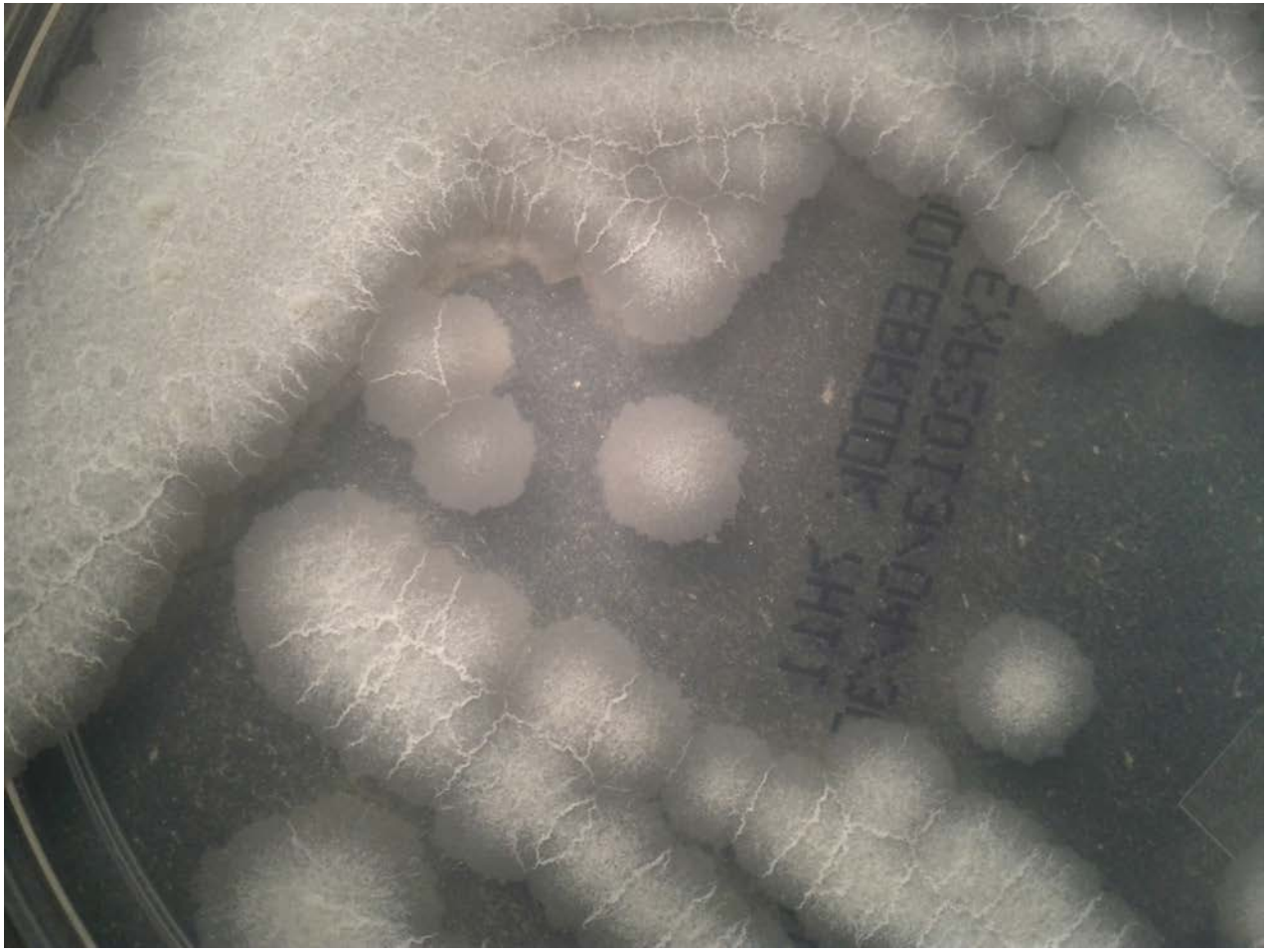
M. abscessus (Rough)



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RGM Medium-1

- Selective Medium (7H9 base + OADC + 4 antimicrobials)
- Sputum samples (N=212) from CF patients (N=172)
- Comparison BCSA vs RGM vs MGIT & LJ
- Direct inoculation onto the plates
- Double decontamination for standard AFB work up (MGIT & LJ)
- Incubation 28d (plates) and 56d (MGIT & LJ)

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RGM Medium-2

	Total	BCSA	AFB	RGM
❖ *M. abscessus* ssp	29	7	18	**28**
❖ *M. chelonae*	5	3	0	**5**
❖ *M. immunogenum*	3	1	0	**2**
❖ MAC	7	0	6	**5**
❖ *M. mucogenicum*	4	0	6	**5**
❖ *M. gordonae*	3	0	0	**3**
❖ **Total**	**51**	**11**	**24**	**47**

Plongla et al J Clin Microbiol May 2017

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Topics

- ❑ Introduction
- ❑ Tool Box 1 & 2
- ❑ Growth Detection
- ❑ **Identification of NTM**
- ❑ Antimicrobial Susceptibility Testing (AST)
- ❑ Strain Characterization

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M. abscessus [rough + smooth] & *M. avium* [translucent]

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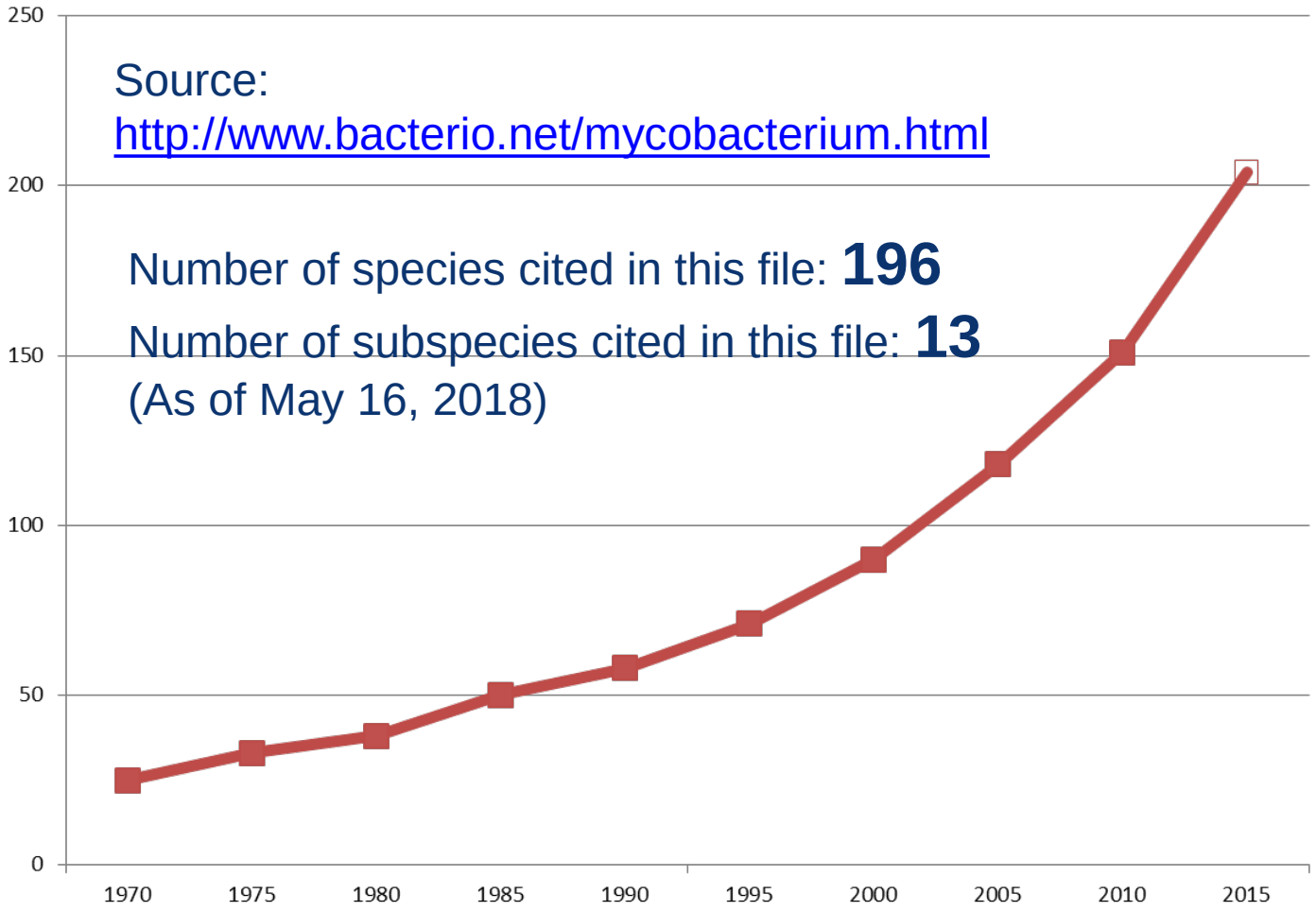
Mycobacterium sp.



Identification

MALDI-TOF MS (Matrix-Assisted Laser Desorption ionization - time of flight mass spectrometry)

MALDI-TOF MS offers a rapid, protein-profiling based technique for identification of mycobacterial isolates from liquid or solid culture media, with high analytical capabilities at a less expensive cost compared to *rpoB* gene sequence analyses.

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MALDI-TOF

MALDI-TOF MS can reliably and rapidly identify

- **approximately 88% of *Mycobacterium* species, 90% of *Nocardia* species, and 51% of other aerobic actinomycetes encountered in routine clinical practice at a tertiary medical center/reference laboratory.**
  - ✓ Using a custom, enhanced library and a streamlined extraction procedure
- Described the ability of the manufacturer's library to identify these groups of organisms and described the effects of lowering the accepted cutoff score from2.0 to 1.7
  - ✓ As the manufacturer continues to expand its database, many laboratories will have the ability to identify many of the isolates they routinely encounter using MALDI-TOF MS.
  - ✓ An expanded custom library may ultimately be the most useful tool for identification of the uncommon species encountered most often in a reference laboratory setting.

Buckwalter et al. J Clin Microbiol. 2016 Feb;54(2):376-84

erm(41) gene variant

Erythromycin Methylase Gene(41)

- Macrolide antibiotics activate the *erm(*41) gene
- Results in inducible (delayed) resistance to clarithromycin and/or azithromycin
- **Mutations** or deletions **inactivate** this gene resulting in **macrolide susceptibility**
- Presence of wildtype or a mutated sequence differs within the 3 subspecies

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erm(41)

***M. massiliense* is positive for the *erm*(41) gene but contains a 273- bp deletion within the gene rendering the gene nonfunctional.**

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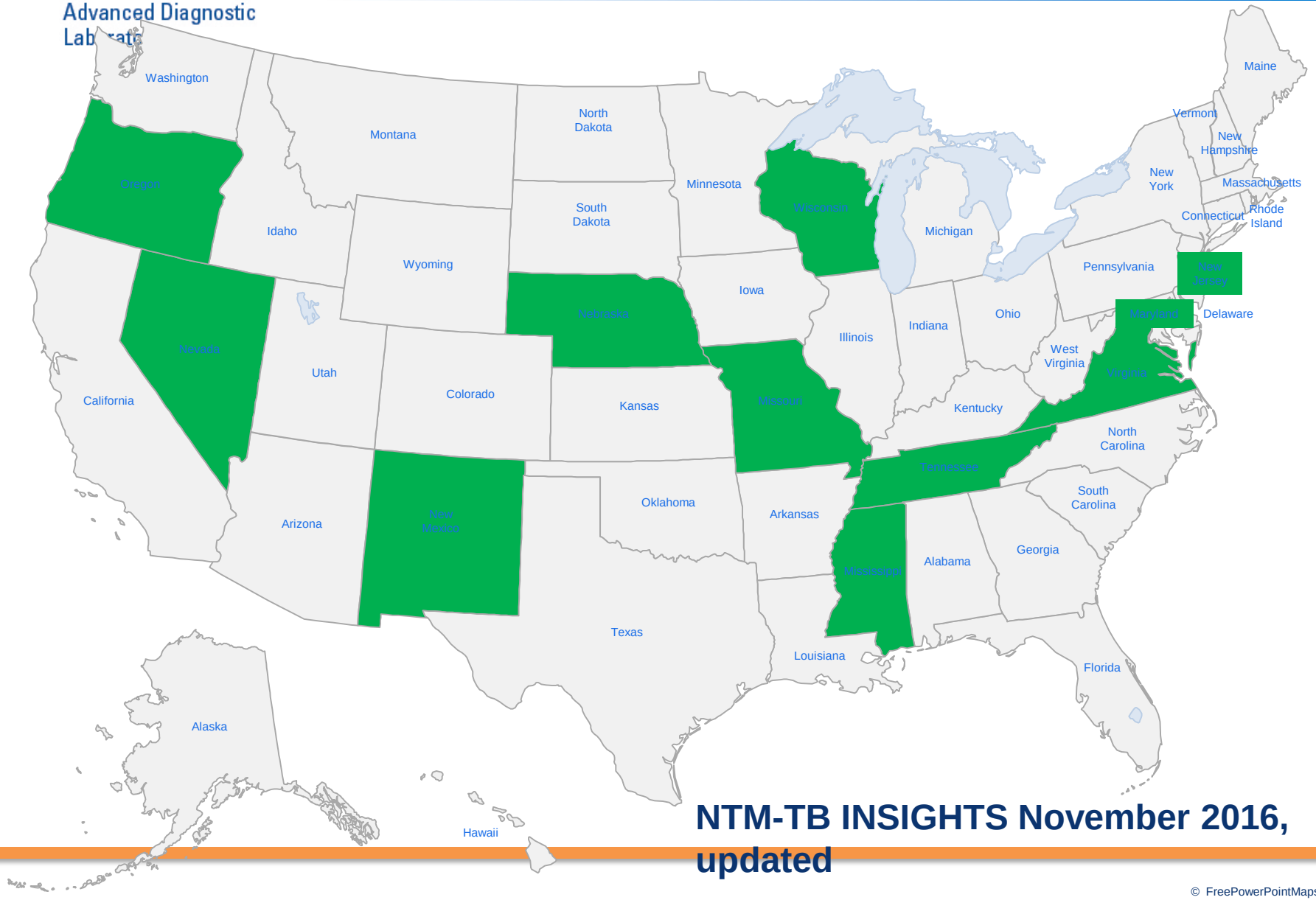
Notifiable NTM by State



NTM-TB INSIGHTS November 2016, updated

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NTM Reporting Requirements

State	Reporting Time for NTM	What is Required to be Reported (websites accessed on 12-11-2016)
Maryland	within one working day	*Mycobacterium* spp., other than *Mycobacterium tuberculosis* complex or *Mycobacterium leprae*
Mississippi	one week	Nontuberculous mycobacterial disease
Missouri	within 3 days	Nontuberculosis mycobacteria (NTM)
Nebraska	within 7 days	*Mycobacteria* spp. (including *M. tuberculosis* complex organisms [for genotyping] and all "atypical" species, to include culture, nucleic acid tests, or positive histological evidence indicative of tuberculosis infection or disease)
Nevada	not specified	Submission of isolates of *Mycobacterium* spp.
New Jersey	within 72 hours	*Mycobacterium*, atypical
New Mexico	within 24 hours	Tuberculosis or other nontuberculous mycobacterial infections (including *Mycobacterium avium* complex or leprosy)
Ohio	close of the next biz. day	Mycobacterial disease other than tuberculosis (MOTT)
Oregon	one working day	Nontuberculous mycobacterial infection (nonrespiratory)
Virginia	immediate	Results of cultures positive for any member of the *Mycobacterium tuberculosis* complex (i.e., *M. tuberculosis*, *M. bovis, M. africanum*) or any other mycobacteria. Results of rapid methodologies, including acid hybridization or nucleic acid amplification, which are indicative of *M. tuberculosis* complex or any other mycobacteria.
Wisconsin	within 72 hours	Mycobacterial disease (nontuberculous)

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- ❑ **Antimicrobial Susceptibility Testing (AST)**
- ❑ Survey 2010 & 2017

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Antimicrobial susceptibility testing

Mycobacterium tuberculosis complex
Mycobacterium avium complex
Mycobacterium kansasii
Mycobacterium marinum
Miscellaneous slowly growing NTM
Rapidly growing mycobacteria (RGM)

CLSI M24-A2 (2011) Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes – Approved standard, second edition (in revision)

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MAC

First-line drugs:

Macrolide (Clarithromycin)

Amikacin

Second-line drugs:

Moxifloxacin

Linezolid

Clofazimine (only MIC, no interpretation)

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RGM

First-line drugs:

Amikacin, cefoxitin, ciprofloxacin,, clarithromycin, doxycycline, imipenem, linezolid, moxifloxacin, trimethoprim-sulfamethoxazole, tobramycin

Second-line drugs:

Tigecycline (only MIC, no interpretation)

Clofazimine (only MIC, no interpretation)

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Antimicrobial susceptibility testing

Rapidly growing NTM

- NTM4: 15-Drug MIC, including Clofazimine/Amikacin Combo
- NTM6: 20-Drug MIC, including Clofazimine/Amikacin Combo

Slowly growing NTM

- NTM10: 10-Drug MIC, incl. RIF-EMB Combo
- NTM9: RIF-EMB Combo, including RIF and EMB single drug MIC

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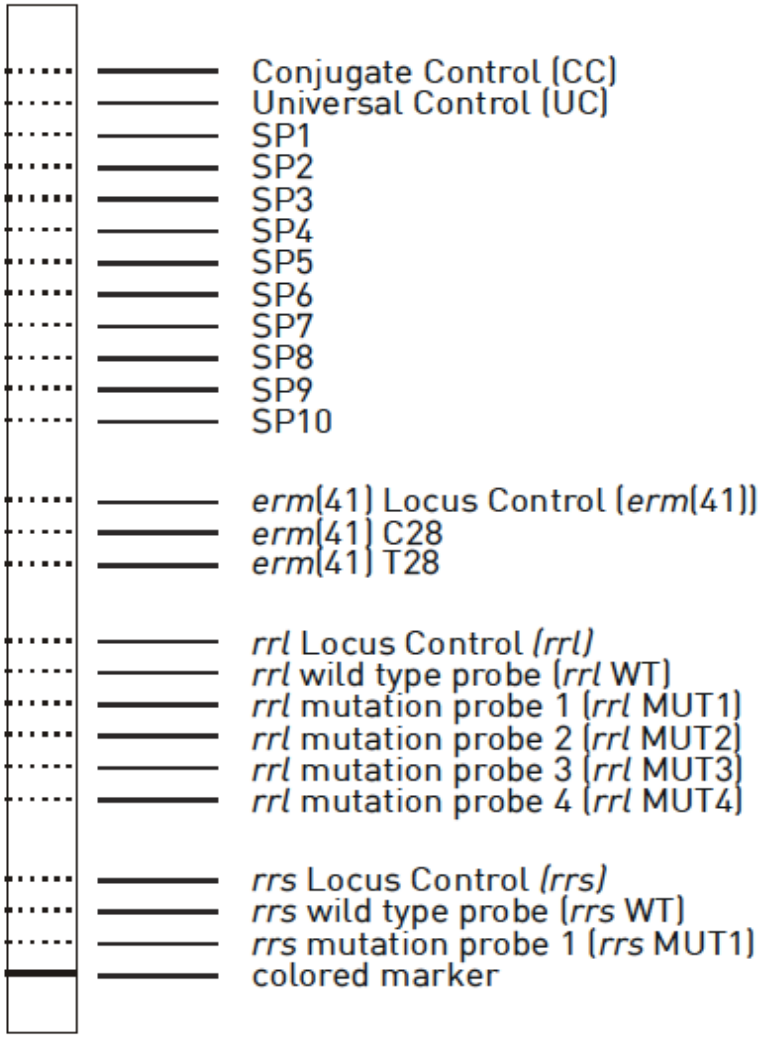
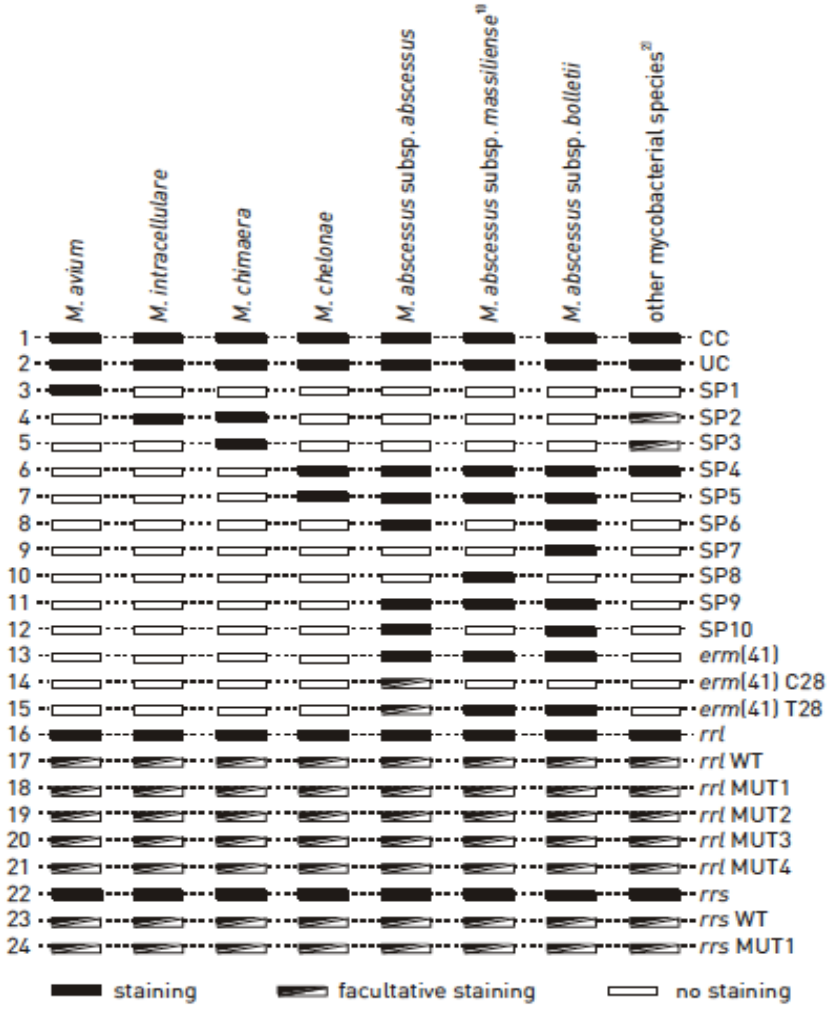
NTM-DR Line Probe Assay





Band No. 1 (CC): Conjugate Control
Band No. 2 (UC): Universal Control

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Identification

	LPA v WGS		
NTM species ID	n (163)	sens †	spec
M. abscessus subsp. *abscessus*	122	100	100
M. abscessus subsp. *massiliense*	36	100	100
M. abscessus subsp. *bolletii*	5	100	100

Table 1: Hain Genotype NTM-DR Ver. 1.0 results compared to whole genome sequencing (WGS) phylogenomic results. sens, % sensitivity; spec, % specificity; n, number of samples in each category. Number of samples for each species (n), Line Probe Assay (LPA). † Sensitivity and specificity are reported in percentages.

Epperson et al NJH: Unpublished Data

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Drug Resistance Markers

	LPA v WGS			
locus	n	sens	spec	n, resistant
16S rDNA (*rrs*) AG	151	100	100	14 (9%)
23S rDNA (*rrl*) MA	156	100	100	3 (2%)
erm(41) MA	127*	100	100	109† (86%‡)

Table 2: Hain Genotype NTM-DR Ver. 1.0 results compared to site-specific extractions of whole genome sequence results for three loci interrogated by the line probe assay, 16S rRNA, 23S rRNA, and erm(41) **M. abscessus* subspp. *abscessus* and *bolletii* only, †T at position 28 of full length *erm*(41) indicates inducible macrolide resistance, ‡In spite of their full length *erm*(41) gene, 14% of samples possessed C at position 28, indicating macrolide susceptibility for these strains

Epperson et al NJH: Unpublished Data

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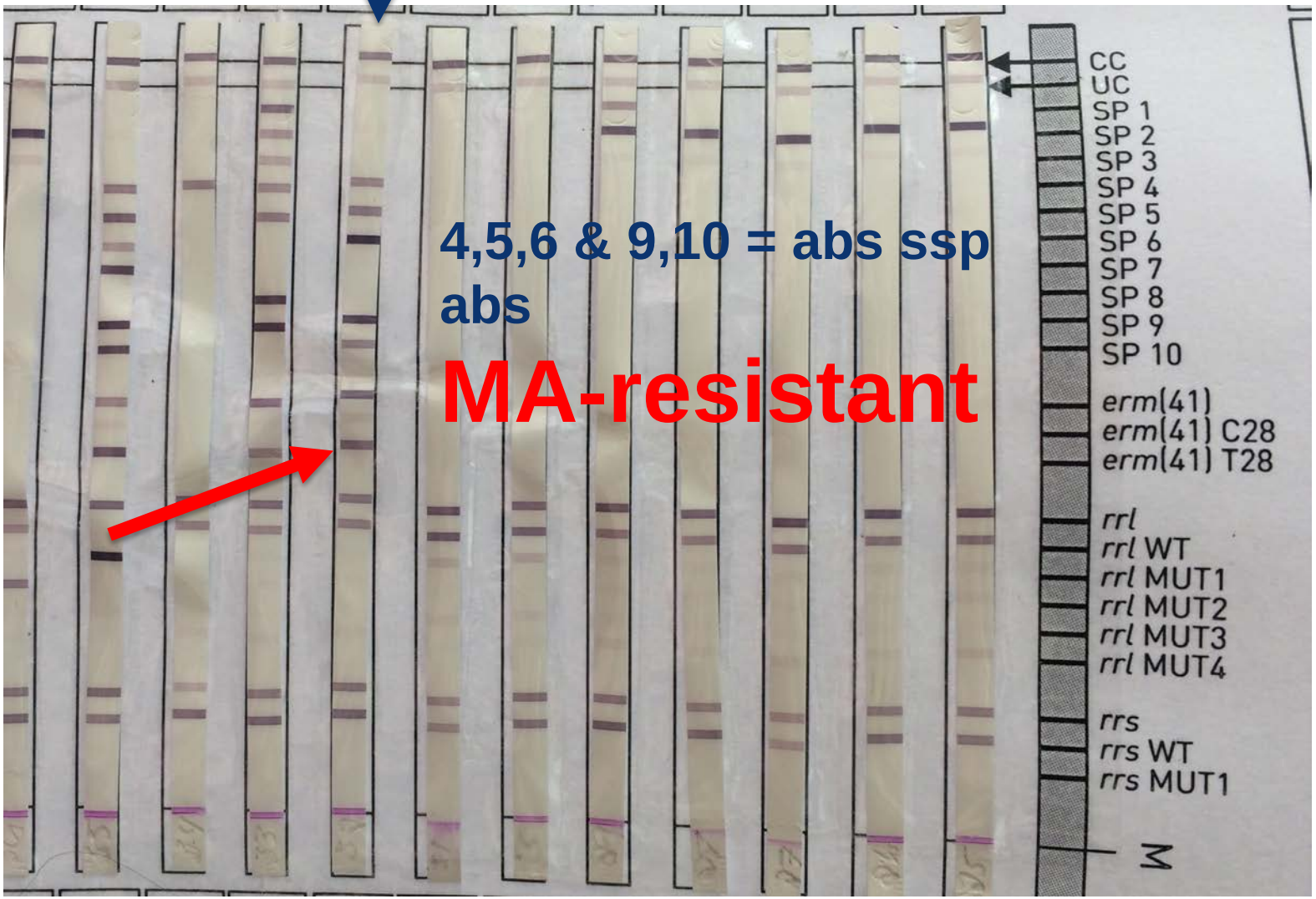
GenoType NTM-DR



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- ❑ **Survey 2010 & 2018**

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APHL Survey 2010

- Distributed to **1,444** laboratories enrolled in mycobacteriology proficiency testing program
- **656 (45%)** responded, 580 performed some level of mycobacteriology service in-house
- **N=580** laboratories
  - 80% hospital-based
  - 9% state public health
  - 4% commercial
  - 4% local public health
  - 3% other

Source:
https://www.aphl.org/programs/infectious_disease/Documents/NationalTBReport_June2012.pdf

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APHL Survey 2010

Culture positivity (**N=212**)

# of labs	NTM-positivity	# of labs	TBC-positivity
8	<1%	72	<1%
164	1-10%	118	1-10%
40	>10%	22	>10%

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APHL Survey 2010

Identification (N=189)

Service	Hospital	State PH	Com.	%
AFB only	1	0	0	0.5
TBC vs NTM	8	1	4	6.9
TBC/some NTM	76	14	8	51.9
TBC/most NTM	25	22	5	27.5
TBC/all NTM	10	13	2	13.2
Total	120	50	19	100

Data not shown for Local PH and other

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NTM Survey 2017

- Distributed to **50** state public health laboratories (10 days ago)
- **50 (100%)** responded
- U.S. population 2016: **323,127,513**

  (D.C. not included)

Unpublished data

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NTM Survey 2017

NAAT Testing (N=50)

	#	%States	%Pop.
No response	1		
No TB NAAT	1		
TB NAAT only	**37**	**74**	
TB + NTM NAAT	**11**	**22**	**17.7**

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NTM Survey 2017

NTM work-up (N=50)

	#	%States
No ID	2	4
In-house all	27	54
In-house some	20	40
Sent to Ref Lab	1	2

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NTM Survey 2017

NTM ID (N=45, multiple responses)

	#	%States
Probes	19	42.2
HPLC	11	24.4
MALDI-TOF*	**17**	**37.8**
Sequencing	**19**	**42.2**
WGS	1	2.2
Other – Line Probes	2	4.4

*Not included: in the near future/validating - 4

NTM Survey 2017

M. abscessus subspecies ID (N=47)

	#	%States	%Pop
Yes	**6**	**12.8**	**28.8**
No	41	87.2	

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NTM-TB INSIGHTS

- **Bi-monthly newsletter**
- **Please feel free to sign up – it is free ☺**

- https://www.nationaljewish.org/for-professionals/newsletters/ntm-tb-insights-newsletter/ntm-tb-insights-sign-up

- Or 

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Thank you!

Maroon Bells, CO