

Massachusetts Department of Public Health
Bureau of Infectious Disease and Laboratory Sciences

It's the Massachusetts Way:
The Use of Maldi-TOF in Identification of
Mycobacteria species

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Objectives

- Snapshot of testing in Massachusetts
 - Emphasis on NTM
- The Massachusetts validation plan
- Efficiencies gained by implementation of the Maldi-TOF

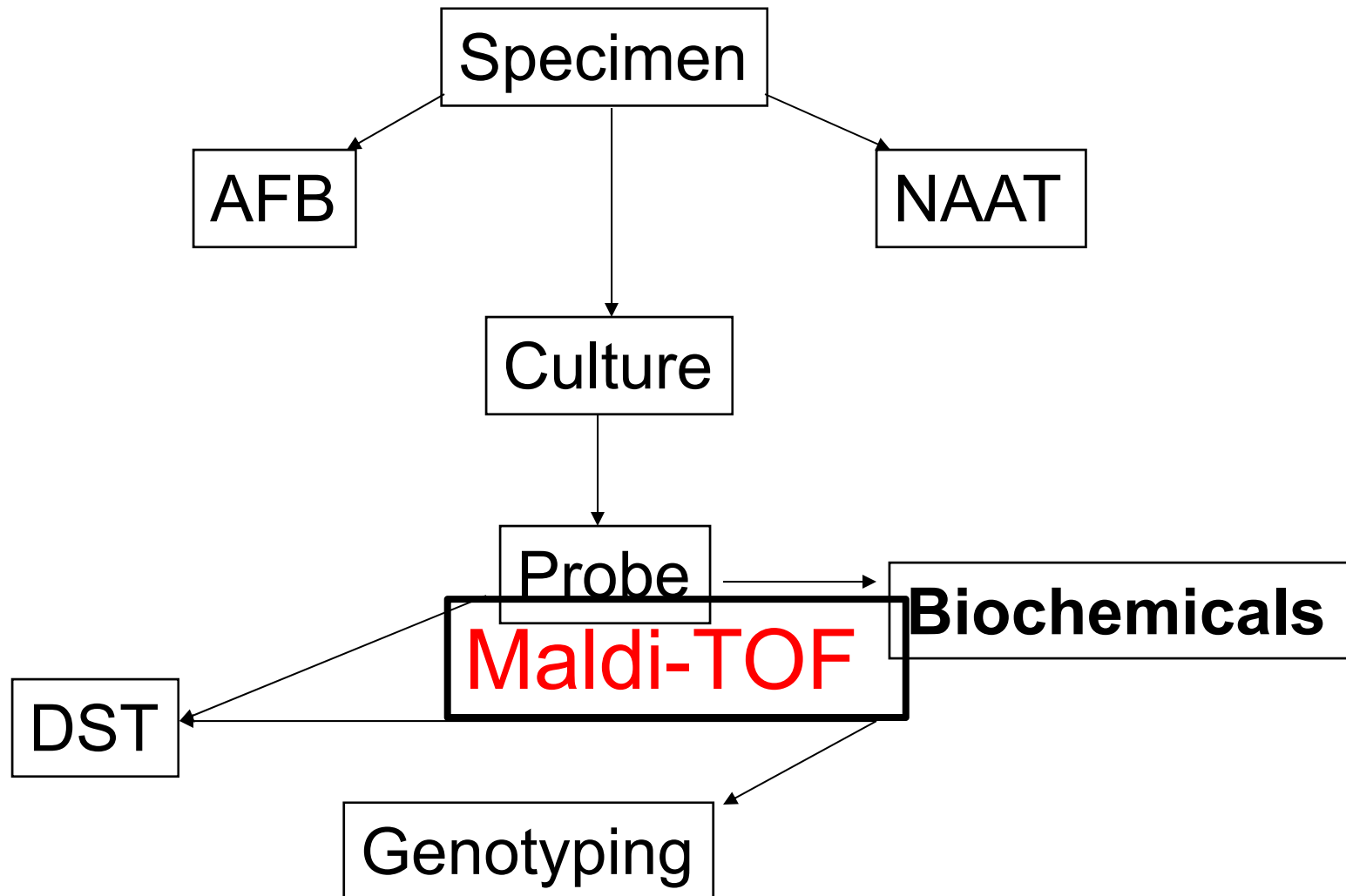
A snapshot in testing...through the years

	2014	2015	2016
Specimens Received	19,335	16,888	16,588
Patients Tested	11,517	9,989	9,692
Patients Positive for Mycobacterium tuberculosis	170	167	157
Patients positive for NTM	857	807	845

Focus on 2014...Pre-Maldi-TOF

Organism Found	2014	
MTBC	165	822
MAC	591	
M. gordonae	66	
M. kansasii	19	71
M. fortuitum	38	
M. Bovis (BCG)	4	
M. xenopi	10	
M. marinum, M. scrofulaceum, szulgai, terrae complex	9	
Probable M. abscessus, but cannot rule out M. chelonae	71	128
Probable M. chelonae, but cannot rule out M. mucogenicum	2	
M. species, Scotochromogen, Photochromogen, Rapid Grower	55	
Total		1021

The Massachusetts Way...of testing for Mycobacteria in 2014



The Massachusetts Way...of adopting the Maldi-TOF

- Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
- to measure a unique molecular fingerprint of an organism



Maldi-TOF vs Probe

Maldi-TOF

- Cheap
- Fast
- Accurate
- Minimal specimen processing time
- Safe
 - Isolate is inactivated
- Can identify many organisms (29 species reported in 2016, 11 in 2014)

AccuProbe

- Time Consuming
- Expensive
- Labor Intensive
- Amplified DNA
- Only used (in MA) for 3 organisms
- If not one of those 3 then we used biochemical ID (which takes even longer)

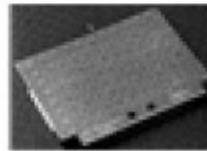
Positive Culture

Extraction of Material

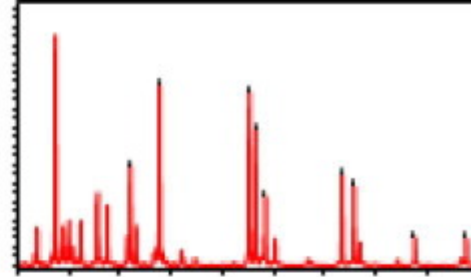
Preparation of target plate

Generate MALDI-TOF profile spectrum

Library Comparison



~20 minutes



The Massachusetts Way...of Designing a Validation Plan

- Viability Study
- Rolling Validation
 - Tested the Species for which we had the highest volume of specimens first
 - Once we reached a high enough percentage of species validated we'd call all of Mycobacteria validated
 - 80%
- Reproducibility Study

Viability Study

- To ensure organism is non-viable when moved to BSL-2 Lab
- 25 Cultures were tested after no heat inactivation, after 30 minutes of heat inactivation and after 60 minutes of heat inactivation

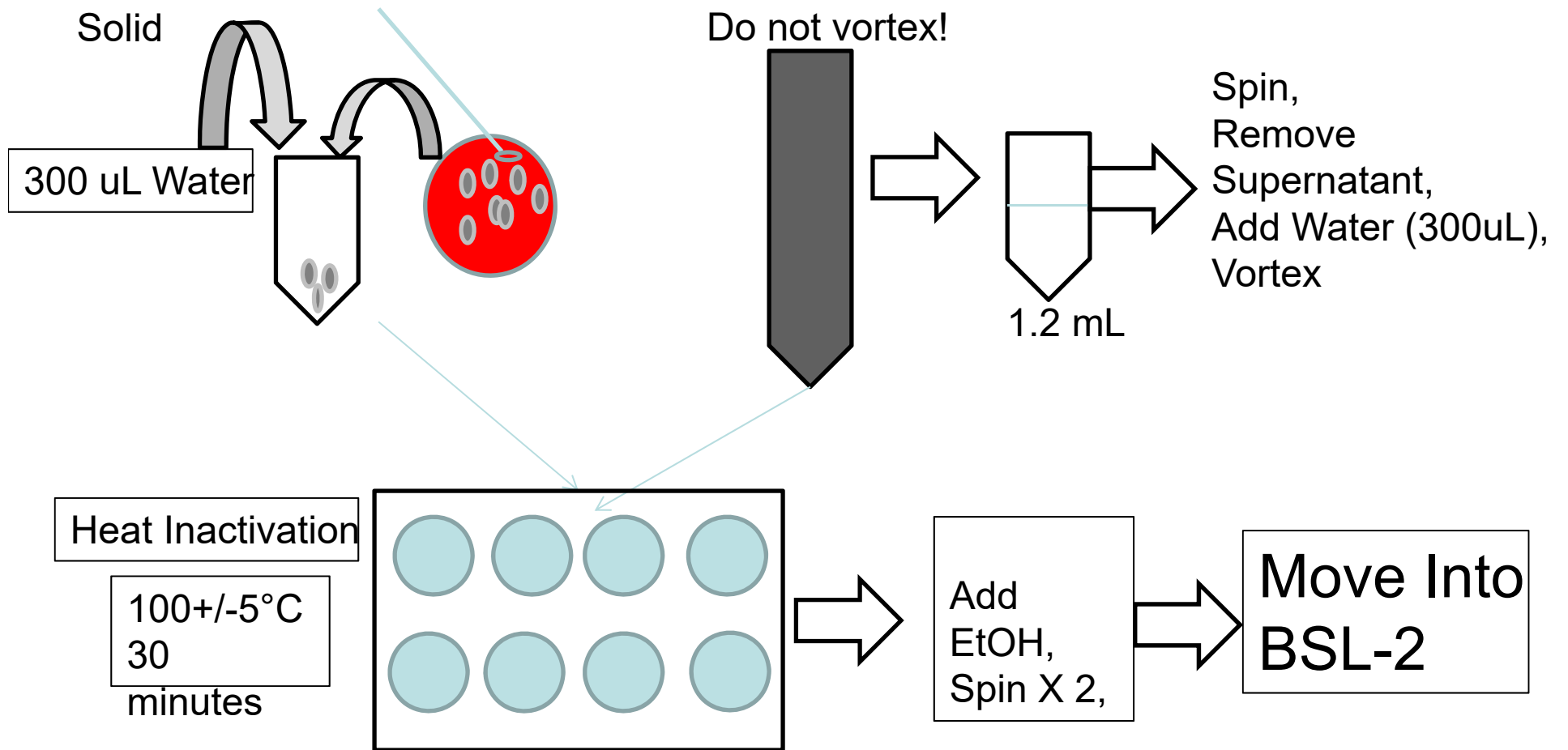
Viability Study Data

Organism	Number of Isolates Tested	#(%) Unheated w/growth:		#(%) Heated at 95-100°C for 30 min w/growth:		#(%) Heated at 95-100°C for 60 min w/growth:	
		In MGIT	On 7H10	In MGIT	On 7H10	In MGIT	On 7H10
M. tuberculosis complex	17	17 (100)	17 (100)	0	0	0	0
M. bovis	3	3 (100)	2 (66)*	0	0	0	0
M. avium complex	1	1 (100)	1 (100)	0	0	0	0
M. kansasii	1	1 (100)	1 (100)	0	0	0	0
M. fortuitum	1	1 (100)	1 (100)	0	0	0	0
Probable M. abscessus	1	1 (100)	1 (100)	0	0	0	0
M. gordonae	1	1 (100)	1 (100)	0	0	0	0
Sterile (water only)	1	0	0	0	0	0	0

Extraction Procedure: BSL-3

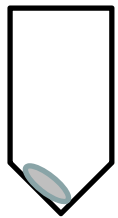
Positive Liquid Culture

Do not vortex!



Extraction Procedure: BSL-2

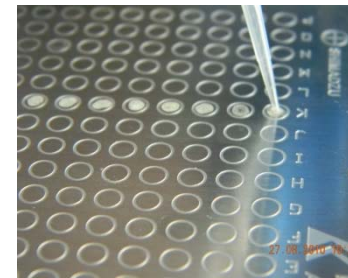
Dry Pellet



Add zirconia/silica
beads
And acetonitrile

Vortex 1 minute

Add Formic Acid
Vortex
Centrifuge



Spot 1 uL on the Plate
Let Dry
Overlay with HCCA Matrix

Validation Data

Reported Organism ID	Maldi ID	Number Tested (Solid)	Number Tested (Liquid)	% Pass
M marinum	M.marinum	3	2	100
M. xenopi	M. xenopi	4	2	100
MTBC	MTBC	13	16	100
M. Gordonae	M. Gordonae	7	13	100
M. kansasii	M. kansasii	9	7	100
MAC	M. avium	19	18	100
	M. chimera-intracellulare	12	14	100
	M. marseillense/ M. chimera-intracellulare	0	1	0
Probable M. abscessus	M. abscessus	21	16	100
	M. chelonae	2	1	*
M. fortuitum	M. fortuitum	5	2	100
	M. peregrinum	1	3	100
	M. mageritense	0	1	**
Probable M. chelonae	M. chelonae	2	1	
	M. mucogenicum phocaicum group	1	1	**
Rapid Grower	M. chelonae	0	1	***
M. gordonae	M. gordonae	13	7	100

Reproducibility Data

	Procedure	Results
Intra-Assay	10 isolates in triplicate	100% Agreement
Same Day Inter- Assay	10 isolates over 2 runs on the same day	100% Agreement
Different Day Inter-Assay	10 isolates over 2 runs on different days	100% Agreement

Initial species Validated on MALDI

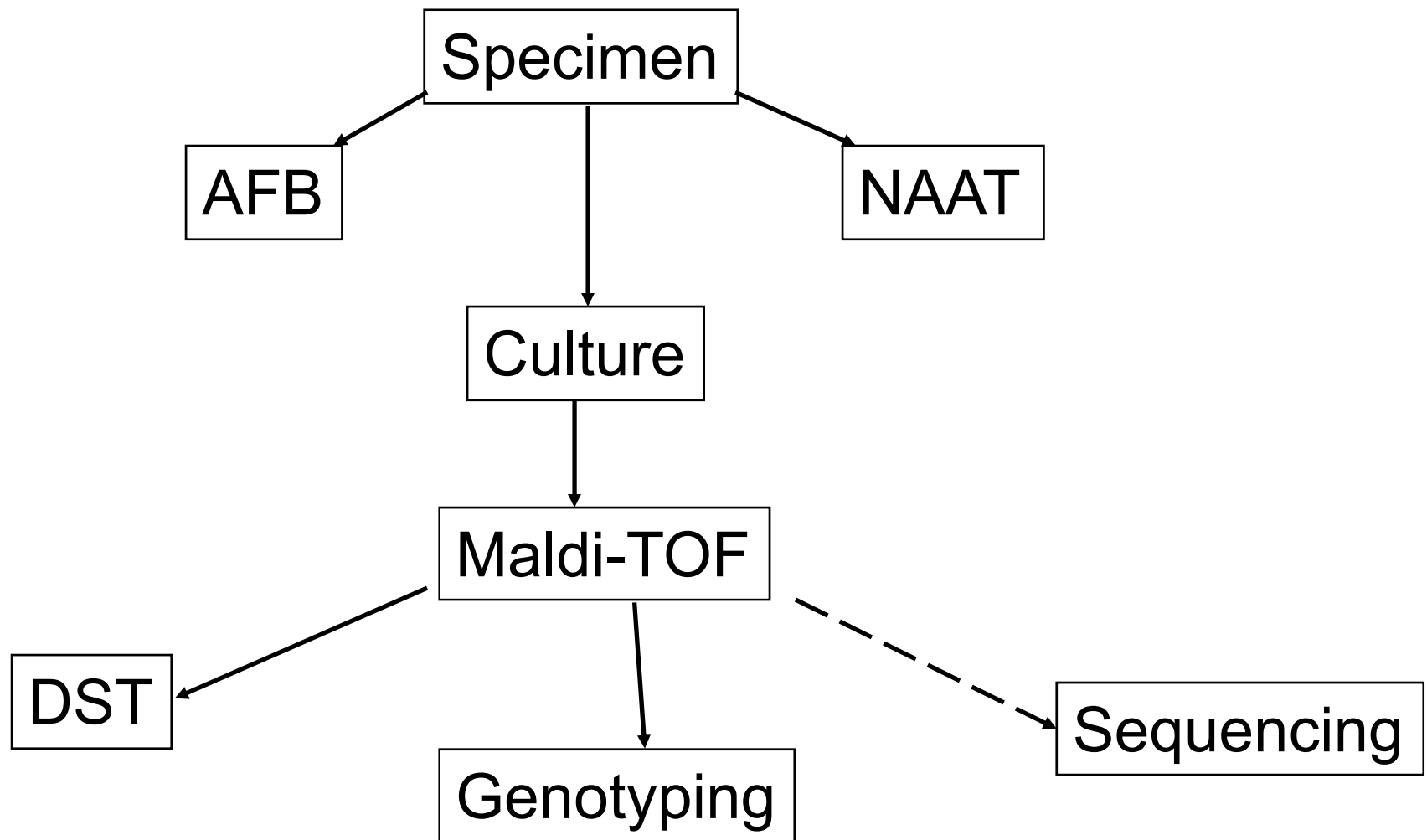
- *M. tuberculosis complex*
- *M. avium complex*
- *M. gordonae*
- *M. kansasii*
- *M. abscessus*
- *M. chelonae*
- *M. marinum*
- *M. xenopi*
- *M. fortuitum/M. fortuitum complex*

Validation Plan

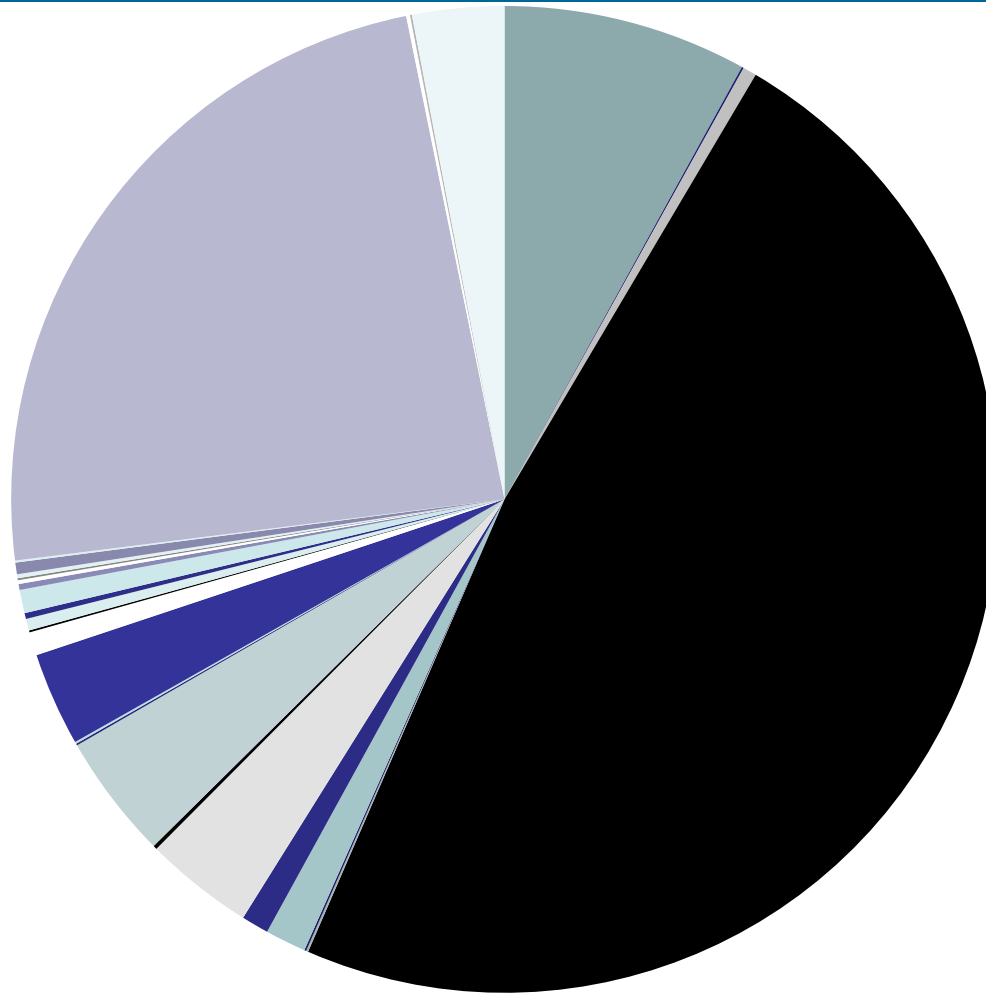


- Viability Study
- Rolling Validation
 - Tested the Species for which we had the highest volume of specimens first
 - Once we reached a high enough percentage of species validated we'd call all of Mycobacteria validated
 - 80%
- Reproducibility Study

The NEW Massachusetts Way...of testing for Mycobacteria

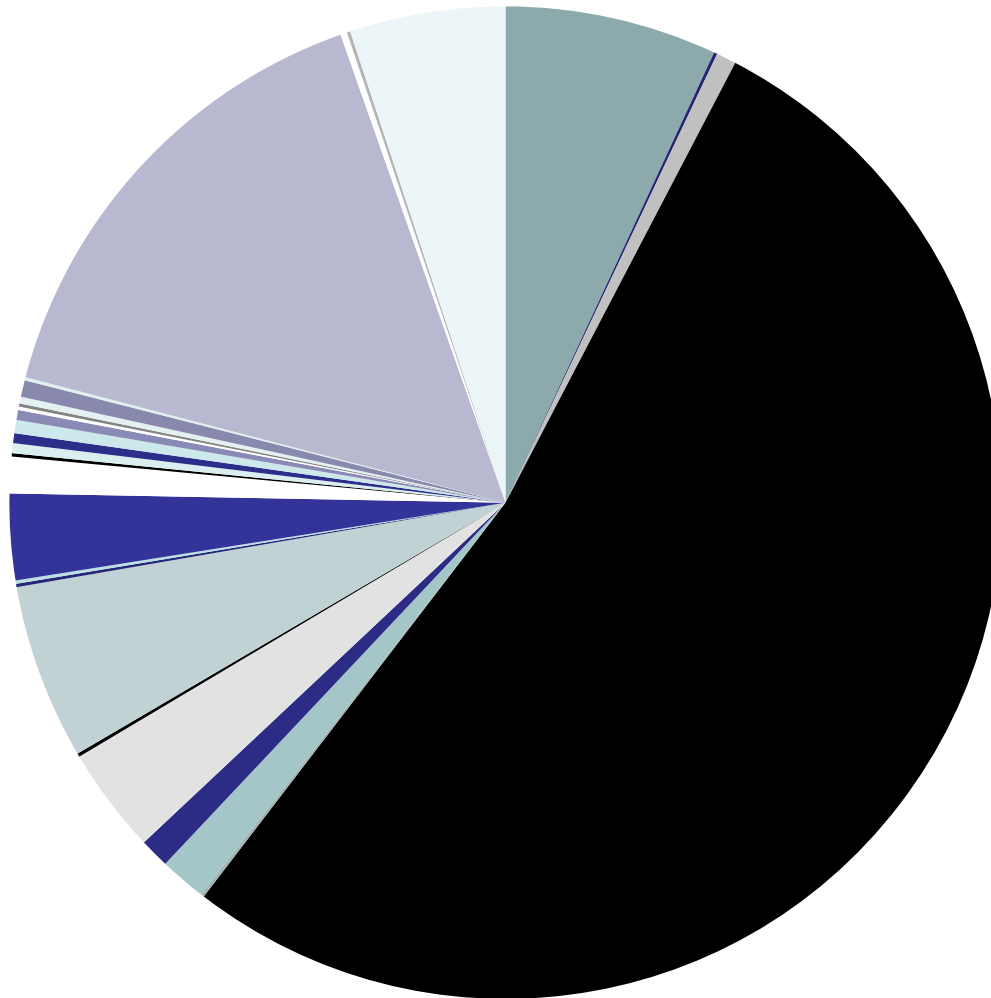


2016 Results by Maldi-TOF



- M. abscessus
- M. arupense
- M. avium
- M. avium complex
- M. bohemicum
- M. brisbanese
- M. chelonae
- M. chimaera intracellulare gp
- M. fortuitum
- M. fortuitum complex
- M. gordonae
- M. haemophilum
- M. immunogenum
- M. kansasii
- M. lentiflavum
- M. liquefaciens
- M. malmoense
- M. marinum
- M. mucogenicum phocaicum group
- M. nebraskense
- M. obuense
- M. parascrofulaceum
- M. peregrinum
- M. simiae
- M. terrae complex
- M. tuberculosis complex
- M. xenopi
- Mycobacterium paragordonae
- Mycobacterium spp.

2016 Maldi Data by Patient



- M. abscessus
- M. arupense
- M. avium
- M. avium complex
- M. bohemicum
- M. brisbanese
- M. chelonae
- M. chimaera intracellulare gp
- M. fortuitum
- M. fortuitum complex
- M. gordonae
- M. haemophilum
- M. immunogenum
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- M. lentiflavum
- M. liquefaciens
- M. malmoense
- M. marinum
- M. mucogenicum phocaicum group
- M. nebraskense
- M. obuense
- M. parascrofulaceum
- M. peregrinum
- M. simiae
- M. terrae complex

The Massachusetts way...of dealing with “No reliable Identification”

- Low Biomass, poor extraction, poor spotting
 - More with liquid culture than positive
 - Liquid Culture tubes flag positive at 10^6
 - Sometimes too low for Maldi
 - ~25%
- Result=“No reliable identification”
- Re-incubate the tube and check visually for sediment for up to 10 days
 - All positive liquid cultures are inoculated into a 7H9 which is usually positive at 5-10 days

The Massachusetts way.... of Reporting

PRELIMINARY REPORT

MICROSCOPY

Report Date: 04/21/2016 Method: Fluorochrome (250x): Acid-fast bacilli not found.

CULTURE

Report Date: 04/25/2016 Acid-fast organisms have been isolated from this specimen. Growth in liquid culture media at 5 days.

IDENTIFICATION

Report Date: 04/27/2016 The acid-fast organism has been identified by MALDI-TOF as *M. abscessus*. If additional organisms are identified an amended report will be issued.
Comment: This assay has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test was adopted for use in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing.

DRUG SUSCEPTIBILITY

DRUG SUSCEPTIBILITY TESTING WILL NOT BE DONE ON THIS CULTURE.

The Massachusetts way.... of Reporting

PRELIMINARY REPORT

MICROSCOPY

The acid-fast organism has been identified by Maldi-TOF as *M. abscessus*

DRUG SUSCEPTIBILITY

DRUG SUSCEPTIBILITY TESTING WILL NOT BE DONE ON THIS CULTURE.

The Massachusetts way.... of Reporting

PRELIMINARY REPORT

This assay has not been cleared or approved by the US FDA.

This was adopted for use and its performance characteristics determined by the Bureau of Laboratory Sciences of the Department of Public Health which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing.

The Massachusetts Way...of lowering costs

Supplies Only	2014	2016
AFB Culture	12.00	12.00
AFB Smear	3.00	3.00
GeneXpert	60.00	60.00
AccuProbe	12.00	N/A
Extra Biochemicals	3.00	N/A
Maldi-TOF	N/A	3.00
Sensitivity (Agar Proportion, SIRE, PZA)	105.00	105.00
Total	195.00	183.00

The Massachusetts Way...of lowering costs

Supplies Only	2014	2016
AFB Culture	12.00	12.00
AFB Smear	3.00	3.00
GeneXper		
AccuProbe		
Extra Bioc		
Maldi-TOF	N/A	3.00
Sensitivity (Agar Proportion, SIRE, PZA)	105.00	105.00
Total	195.00	183.00

**\$12.00 per culture X
1500 cultures =
\$18,000/year in savings**

Final Considerations

- Maldi-TOF is expensive
 - \$150-\$200K
 - Gain efficiency by using in multiple sections
 - Annual Service Contract can be up to \$30K
- Biosafety concerns if you have it in a BSL-2 Lab
 - Viability study is important
 - Thorough Risk Analysis

The Massachusetts Way....

I'm not
telling you
it's going
to be

easy,

I'm
telling you
it's going
to be

WORTH IT.

Questions?

