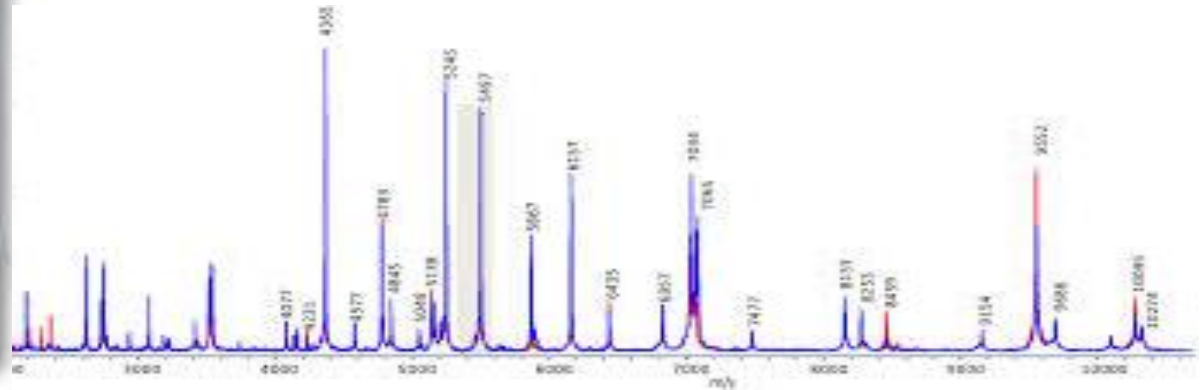




Update on MALDI-TOF Validation

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WMLN 2019



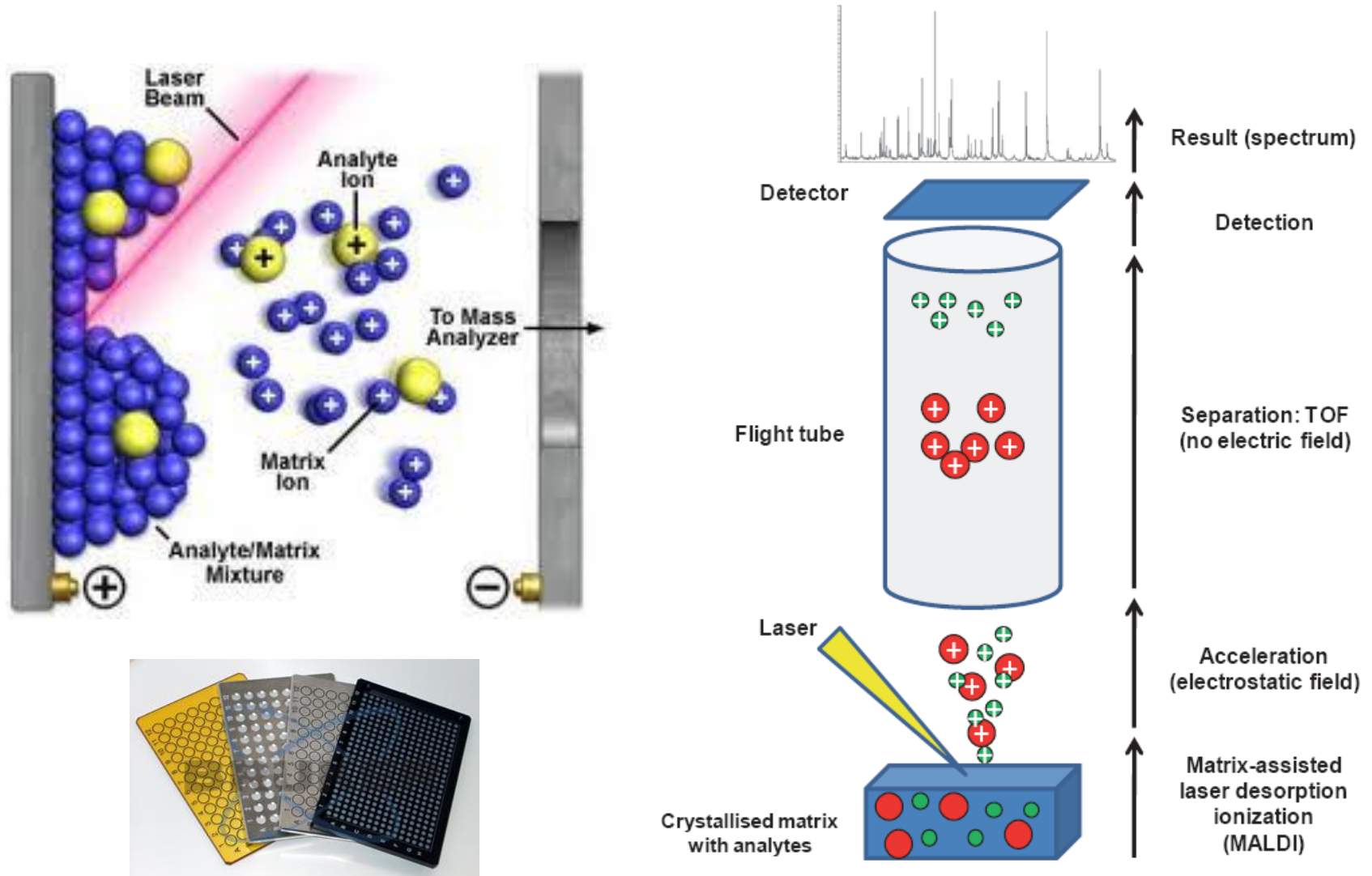


Matrix-Assisted Laser Desorption Ionization Time-of-Flight

- The instrument consists of a platform, a tube, a laser and a detector
- Used to analyze proteins and other biomolecules.
- Separation of such molecules based on mass to charge ratio occurs along TOF tube as molecules travel to detector.
- Dedicated software analyzes mass spectra against stored profiles and gives microorganism identification



MALDI-TOF Ionization

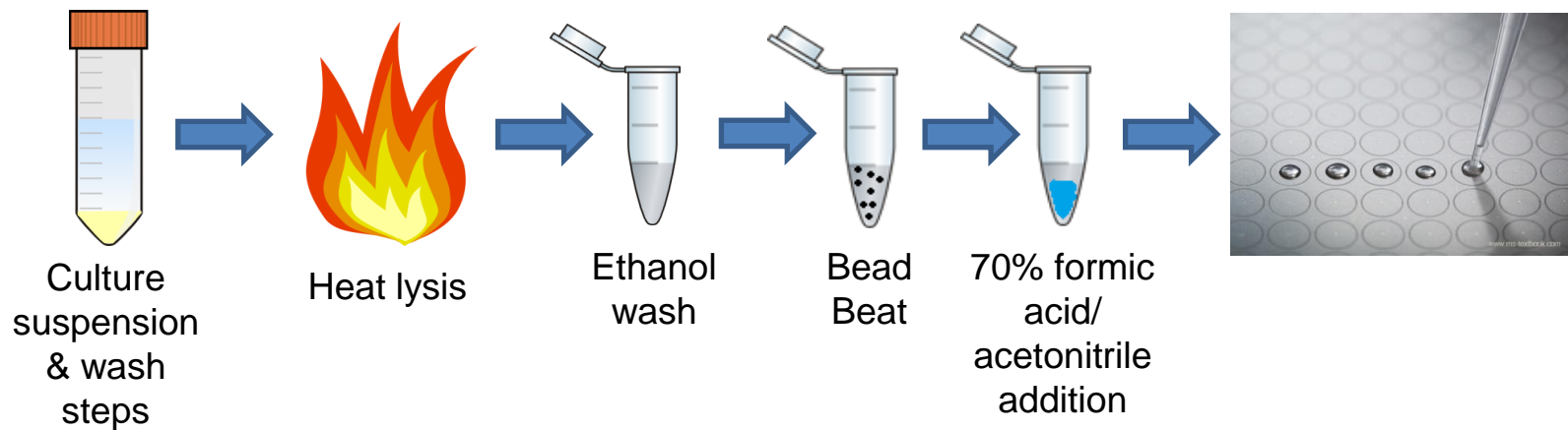




- Bruker Microflex LT
- RUO library
 - Myco Version 5
- MBT compass software



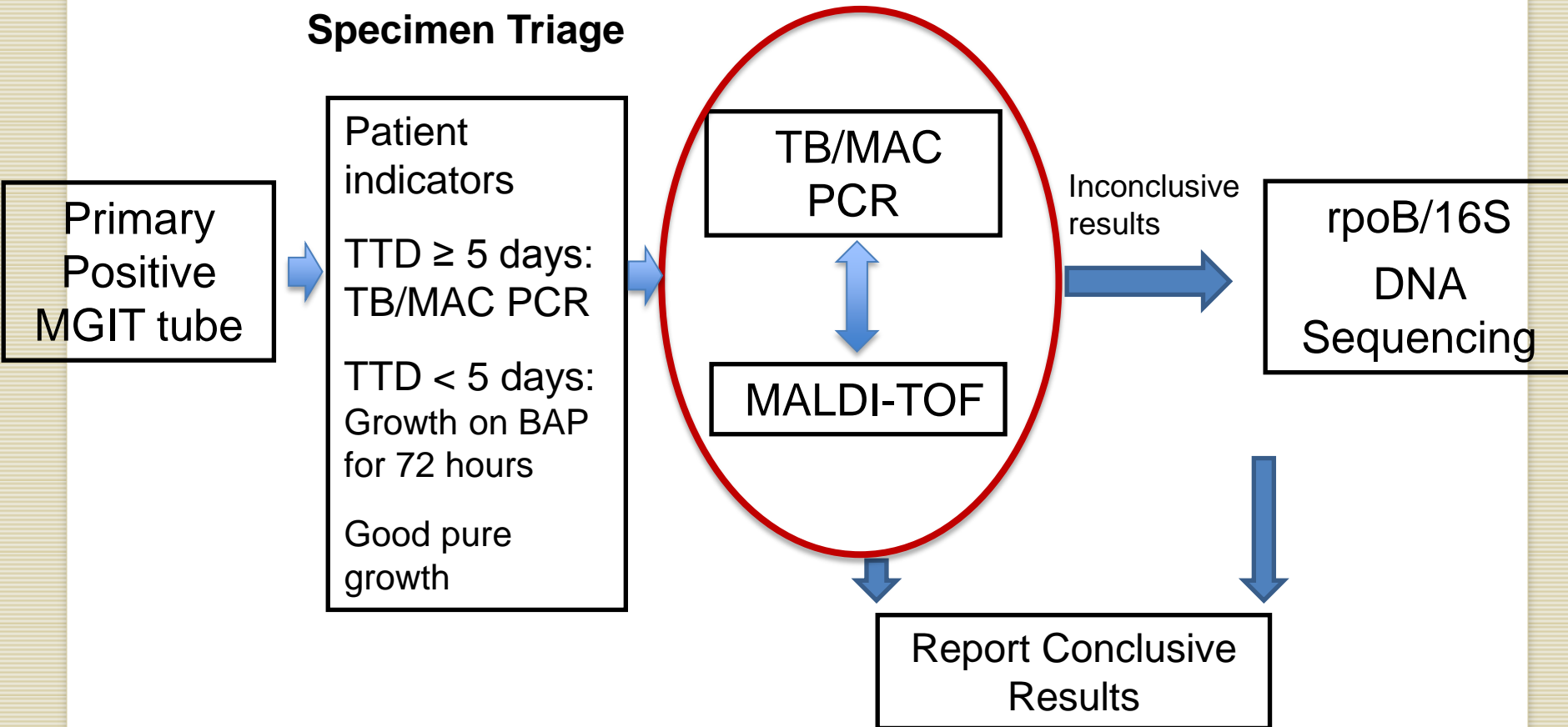
- WSLH uses a modified version of the Bruker MycoEX extraction method.
 - Includes an additional wash step for liquid media.
 - Combined Acetonitrile / Formic acid step.





WSLH Algorithm for Primary Positive MGIT Tubes

Specimen Triage





Ongoing species validation

- Continue to validate less common mycobacteria species as they are isolated.
- Before MALDI results can be reported for each species, confirmatory testing of at least three unique strains is performed by MALDI and at least one other confirmatory method including real-time PCR, 16S, and *rpoB* DNA sequencing.



Fully validated species

M. abscessus

M. arupense

M. asiaticum

M. avium complex

M. chelonae

M. fortuitum

M. franklini

M. goodii

M. gordonae

M. immunogenum

M. kansasii

M. kumamotoense

M. lentiflavum

M. magrebitense

M. malmoense

M. marinum

M. mucogenicum/phocaicum

M. nebraskense

M. neoaurum

M. paragordonae

M. peregrinum

M. porcinum

M. scrofulaceum

M. septicum

M. simiae

M. smegmatis

M. szulgai

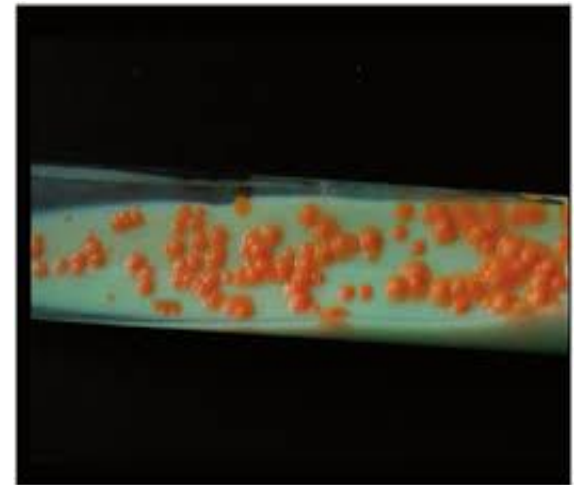
M. tuberculosis complex

M. xenopi



M. gordonae validation

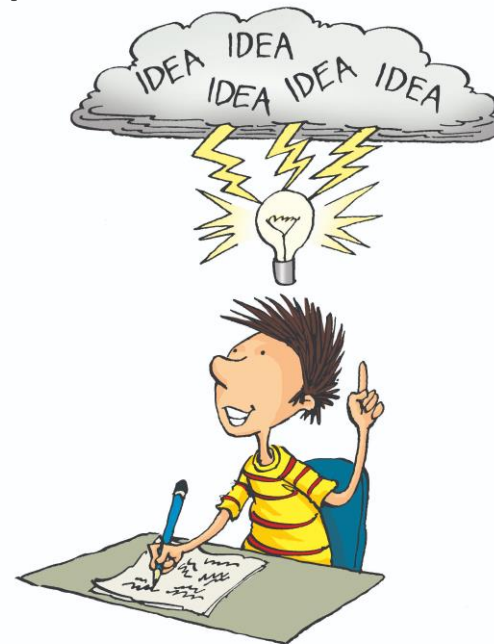
- Validated reporting threshold is 1.8 for mycobacteria.
- Sometimes difficult to reach reportable score for *M. gordonae*.
- Ended up performing MALDI repeatedly and/or sequencing.





M. gordonae validation

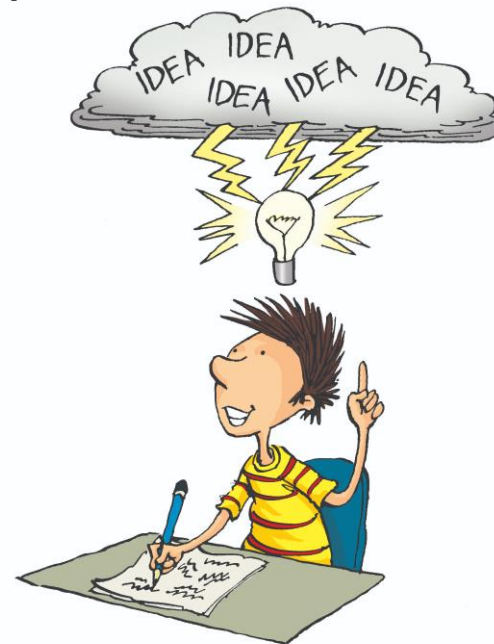
- Options to solve the problem?





M. gordonae validation

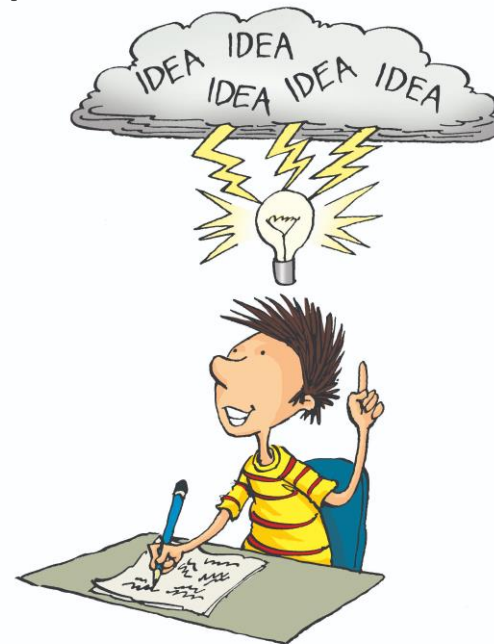
- Options to solve the problem?
 - Change extraction?





M. gordonae validation

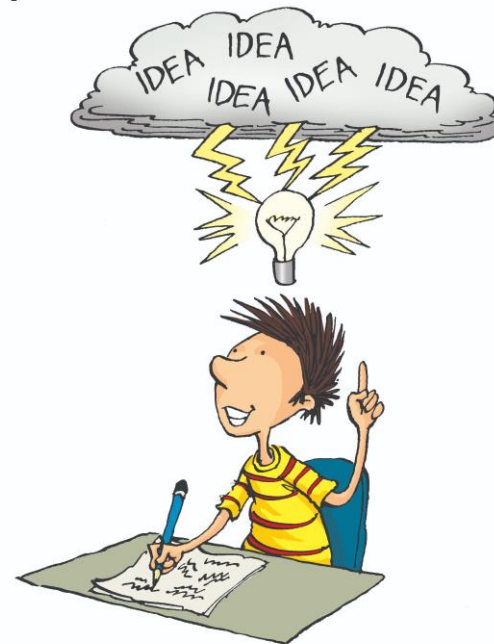
- Options to solve the problem?
 - Change extraction?
 - Create *M. gordonae* library ?





M. gordonae validation

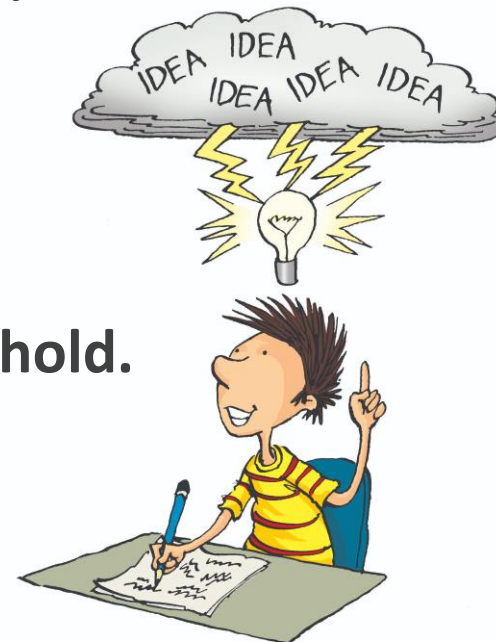
- Options to solve the problem?
 - Change extraction?
 - Create *M. gordonae* library ?
 - CDC Microbenet ?





M. gordonae validation

- Options to solve the problem?
 - Change extraction?
 - Create *M. gordonae* library ?
 - CDC Microbenet ?
 - **Validate lower reporting threshold.**





M. gordonae validation

- Performed retrospective analysis.
- Found 30 isolates between 12/16 – 9/18 that gave MALDI ID of *M. gordonae* with score 1.7-1.799
- 30/30 were confirmed to be *M. gordonae* by MALDI repeat or gene sequencing.
- Validated a lowered reporting threshold to **1.7**



Nocardia Validation

- Validated MALDI for identification of *Nocardia*, using mycobacteria extraction protocol.
- Analyzed 21 patient isolates and 8 QC organisms



- Compared MALDI ID to sequencing result & Known ID(QC organisms).
 - *N.nova*(10/10)
 - *N.cyriacigeorgica* (5/5)
 - *N. farcinica* (4/4).



Nocardia Validation

- There were various discrepancies between MALDI vs. sequencing results for less common species.
- Report *N.nova*, *N.cyriacigeorgica*, and *N.farcinica* to species, score ≥ 1.8 .
- Report all other *Nocardia* results as *Nocardia species*, score ≥ 1.7 .

Specimen	MALDI Date	MALDI ID	MALDI Score		Sequencing result	
16mm3923	11/21/2017	<i>Nocardia veterana</i>	2.281	2.228	<i>Nocardia</i> sp. Sp080513KE-16	99%
16mm5009	11/21/2017	No peaks	0	0	<i>Nocardia exalbida</i>	100%
16mm5010	11/21/2017	No peaks	0	0	<i>Nocardia exalbida</i>	99%
17mm4709	12/7/2017	<i>Nocardia araeensis</i>	1.784	1.79	<i>Nocardia asteroides/ asiatica/ abscessus</i>	99%
Specimen					ID(QC organism)	
BQC-006-001	1/9/2018	<i>Nocardia otitidiscaviarum</i>	1.976	2.039	<i>Nocardia otitidiscaviarum</i>	
BQC-006-004	1/9/2018	<i>Nocardia brasiliensis</i>	1.701		<i>Nocardia brasiliensis</i>	
BQC-006-015	1/9/2018	<i>Nocardia</i> sp	1.745	1.82	<i>Nocardia brasiliensis</i>	
BQC-006-019	1/9/2018	<i>Nocardia wallacei</i>	1.673	1.863	<i>Nocardia transvalensis</i> com.	



Manual analysis

- Occasionally protein peaks will be too low in intensity to be captured by the automatic acquisition run.
- even when they appear to be good spectra while observing the run in real-time.
- An alternate “Manual analysis” technique can be used.
 - Flex Control
 - Compass explorer(offline client)



Flex Control

Manually add and save spectra

File Display View Tools Compass Help

Clear Sum Start Save Undo Add Save As...

Shots: 40 / 40 Added: 0 Freq: 60.0 36 %

Spot: C10.0 Geometry: MSP 96

Carrier: G_93A21F43_7714_435C_8F0B688246C9A29B

Method: MBT_FC_par

AutoXecute Sample Carrier Detection Spectrometer Processing Calibration Setup Status

Calibration strategy: Mass Control List MBT_Standard

Name	Ref. Mass/Da	Cur. Mass/Da	Err/ppm	Err/Da
• RL29 [M+2H] ²⁺	3637.80000			
• RS32 [M+H] ⁺	5096.80000			
• RS34 [M+H] ⁺	5381.40000			
• RS33meth [M+...]	6255.40000			
• RL29 [M+H] ⁺	7274.50000			
• RS19 [M+H] ⁺	10300.10000			
• RNAse A [M+H] ⁺	13683.20000			
• Myoglobin [M+...]	16952.30000			

Zoom Range: ± 5.0 %

Peak Assignment Tolerance

From Mass Control List

Near Neighbor (50 ppm)

User Defined 1000 ppm

Mode: Quadratic

Calibrants Std dev (ppm): Before Last Fit In Last Fit Properties...



Compass Explorer

Add Spectra

Add Libraries

Name	Description	Creation Date
Listeria	contains 11 MSPs	12/16/2007 7:47:21 PM
Mycobacteria	contains 880 MSPs	7/13/2018 12:23:48 AM
IVD	contains 7712 MSPs	2/28/2019 8:52:09 PM
Mycobacteria Library file	contains 912 MSPs	8/10/2018 8:55:31 PM
SR	contains 104 MSPs	12/18/2018 9:27:32 PM
SR BBFV	contains MSPs of the foll...	12/18/2018 9:27:32 PM
Filamentous Fungi	contains 468 MSPs	12/18/2018 10:27:30 PM
BDAL	contains 7854 MSPs	2/28/2019 8:52:09 PM

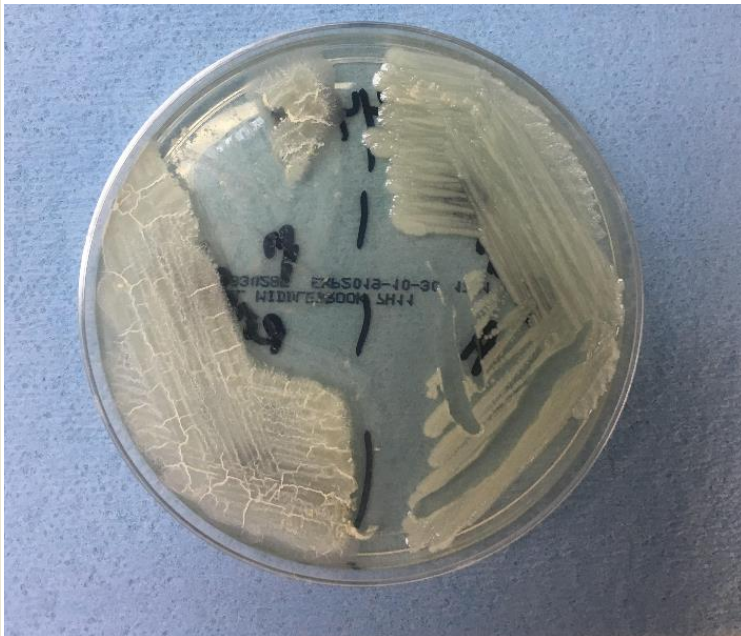
Select spectra and press <Ctrl - G> to view real spectra or <Ctrl - N> for normalized spectra.

Selected MSP Current MSP



Extended Direct: Rapid Growers

- Could MALDI extended direct method be used to rapidly test multiple colony types often present in Rapid Grower cultures?





Extended Direct: Rapid Growers

- **Extended Direct:**

- Transfer isolated colony to target with a toothpick.
- Overlay with 1 μ l of formic acid & 1 μ l of matrix
- Air dry and analyze.





Extended Direct: Rapid Growers

- Performed initial side-by-side of Myco extraction vs. Extended direct.

Specimen	Mycobacteria tube extraction method			Extended direct method		
	MALDI score	MALDI score (MANUAL)	MALDI ID	MALDI score	MALDI score (MANUAL)	MALDI ID
19mm2308	2.00		M. septicum	0.00	2.00	M. septicum
19mm2267	0.00	2.30	M. fortuitum grp	2.20		M. fortuitum group
19mm1942	2.170		M. abscessus	0.00	1.95	M. abscessus
19mm1774	2.140 2.08		M. abscessus	0.00	2.14	M. abscessus
19mm1896	1.930 1.88		M. chelonae	0.00	1.38	NO ID
19mm1890	1.810		M. chelonae	0.00	1.74	M. salmoniphilum
ATCC700686	2.070 1.94		M. pergrinum	0.00	1.57	NO ID
19mm1921	2.220 2.25		M. pergrinum	0.00	2.15	M. peregrinum
19mm1895	0.00	2.11	M. mucogenicum grp	0.00	2.08	M. mucogenicum grp
19mm1775	2.230 2.22		M. mucogenicum grp	1.770 1.88		M. mucogenicum grp



Extended Direct: Rapid Growers

- Performed extended direct on 22 previously identified isolates
 - *M. abscessus*: 3/4 isolates gave good ID, 1 gave a score below or reporting threshold.
 - *M. fortuitum*: 3/3 gave good ID
 - *M. mucogenicum* group: 3/3 gave good ID
 - *M. peregrinum*: 3/4 gave good ID, 1 gave no result
 - *M. chelonae*: 8/8 isolates gave low score or no result



Remaining Discrepancies

- Cannot distinguish between members of the MTB complex.
- Cannot Identify *M. abscessus* to subspecies level.
- “Mass spectra of *Mycobacterium fortuitum* complex members are similar to each other; especially mass spectra of *M. farcinogenes*, *M. fortuitum* ssp *fortuitum*, *M. porcinum* and *M. senegalense*.”