Frequency of Mixed *Mycobacterium* Species Infections Detected by the INNO-LiPA MYCOBACTERIA v2 Hybridization Line Probe Assay January 2015 to October 2016

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Abstract

Objective: To evaluate the frequency of mixed mycobacteria infections detected from 2015 to 2016 using the Fujirebio (Ghent, Belgium) manufactured INNO-LiPA MYCOBACTERIA v2 line probe hybridization assay (LPA), which identifies 18 clinically relevant *Mycobacterium* species.

Study Design: PCR is used to amplify the 16S-23S internal transcribed spacer region of *Mycobacterium* species. This biotinylated amplified material is reverse hybridized with specific oligonucleotide probes immobilized on membrane strips. A final colorimetric step produces characteristic bands that are associated with specific *Mycobacterium* species. More than one *Mycobacterium* species can be detected on a single strip. Twenty-two months of data (January 2015 through October 2016) was assessed for the number of specimens where more than one species of mycobacteria was detected by the LPA, compared to the total number of LPA positive specimens.

Results: During the study period, a total of 1,430 LPA identifications were performed, of which the LPA detected 3% (40) mixed mycobacterial infections. Most of the mixed populations were *M. avium complex* with *M. intracellulare* infections followed by *M. avium complex* with *M. abscessus* infections. Other clinically relevant mixed infections were also detected in this study such as *M. tuberculosis complex* with *M. avium complex* in far lower numbers but nonetheless considered significant for patient care.

Conclusions: In Minnesota (MDH TB Lab), mixed mycobacteria infections do occur with low frequency, which can be easily and rapidly detected using the INNO-LiPA MYCOBACTERIA v2 hybridization line probe assay. Accurate mycobacterial identification and detection of mixed infections is essential for not only epidemiological investigations but for correct diagnosis and appropriate patient treatment.

1. Interpretation Chart: Banding Patterns on INNO-LiPA Probe Strips

- **Assay can detect 18 clinically relevant *Mycobacterium* spp.**

Line	Probe Names	*Mycobacterium sp.*	MTBC	*M. kansasii*	*M. kansasii*	*M. kansasii/gastri*	*M. xenopi*	*M. gordonae*	*M. genavense*	*M. simiae*	*M. marinum/M. ulcerans*	*M. celatum*	MAC	MAC	*M. intracellulare*	*M. intracellulare*	*M. scrofulaceum*	*M. malmoense*	*M. haemophilum*	*M. chelonae*	*M. abscessus*	*M. chelonae*	*M. fortuitum* complex	*M. smegmatis*
1	Conj ctrl	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	MYC genus	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	MTB		3																					
4	MKA-1			4																				
5	MKA-2				5																			
6	MKA-3					6																		
7	MXE						7																	
8	MGO							8																
9	MGV								9															
10	MSI									10														
11	MMU										11													
12	MCE											12												
13	MAIS												13	13	13	13	13	13	13*					
14	MAV													14										
15	MIN-1														15									
16	MIN-2															16								
17	MSC																17							
18	MML																	18						
19	MHP																		19					
20	MCH-1																			20	20	20		
21	MCH-2																				21			
22	MCH-3																					22		
23	MFO																						23	23
24	MSM																							24

2. AFB (+) Culture Workflow

Positive Culture
MGIT broth, 7H11 agar, LJ slant, Blood culture bottle, etc.

↓

Prepare & read Kinyoun Acid Fast Stain

↓

AFB Present

- ← Previous (+) *Mycobacteria* ID within 2 months
  - ↓ **Subculture to solid media**
    - Visually inspect growth weekly
    - If morphology correlates to most recent LPA ID, report phenotypic observed growth *i.e. Morphology consistent with Mycobacterium avium complex*
- → No previous positive culture or (+) *Mycobacteria* ID >2 months ago
  - ↓ **Subculture to solid media**
    - Run LPA
    - Visually inspect growth weekly on solid media
    - Correlate growth with LPA result
  - ← No I.D. with LPA, reflexed to16S sequencing

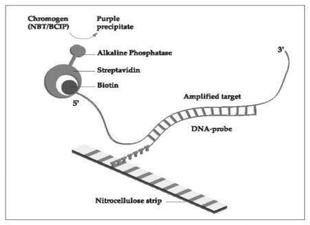
3. INNO-LiPA Line Probe Assay Methods

Step 1: Amplification of 16S-23S rRNA spacer region

Step 2: Hybridization & stringent wash of amplified 16S-23S spacer region with specific probes immobilized on strip

Step 3: Color development of membrane strips

Step 4: Interpretation of signal pattern



Example of line probe assay (LPA) detection system based on the reverse hybridization principle.

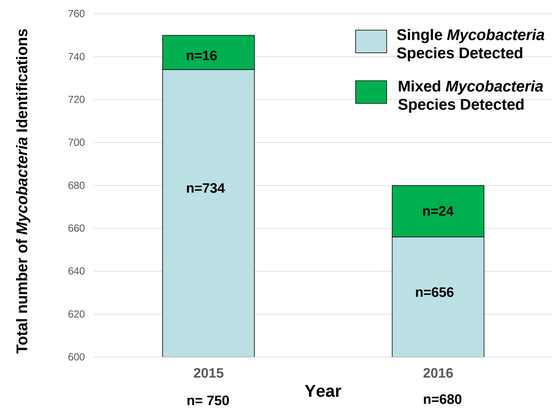
Auto-LiPA 48: Semi-automated test performance from hybridization to color development.

INNO-LiPA MYCOBACTERIA v2
Data Reporting Sheet

1. *M. avium complex*
2. *M. avium complex*
3. *M. avium complex* + *M. chelonae*
4. *M. intracellulare* + *M. gordonae*
5. *M. gordonae*
6. *M. fortuitum complex*
7. *M. fortuitum complex*
8. Not *Mycobacterium* species

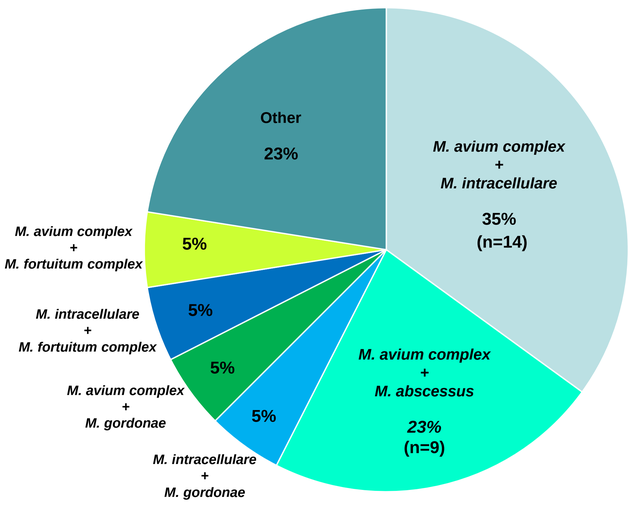
4. Total Line Probe Identifications

January 2015 - October 2016



5. Mixed *Mycobacterium* spp. Detected

January 2015 - October 2016



Other (n=1 each): *M. tuberculosis complex + M. gordonae*
M. tuberculosis complex + M. avium complex
M. abscessus + M. fortuitum complex
M. abscessus + M. intracellulare
M. avium complex + M. simiae
M. intracellulare + M. simiae
M. intracellulare + M. chelonae
M. fortuitum complex + M. gordonae
M. avium complex + M. intracellulare + M. abscessus

6. Conclusions

- The INNO-LiPA MYCOBACTERIA v2 hybridization line probe assay (Fujirebio) serves as our primary means of identification.
  - One strip replaces multiple tests; easy to perform
  - Detection of 18 clinically relevant mycobacterial species
  - Applicable to early liquid culture and solid media
  - Quick visual interpretation
  - Mostly automated using the Auto-LiPA 48
  - **Mixed populations easily identified**
- Mixed mycobacteria infections are detected in the MN TB Laboratory at a frequency of 3%, with *M. avium complex* + *M. intracellulare* (35%) and *M. avium complex* + *M. abscessus* (23%) being the two most common mixed populations.
- Accurate and rapid laboratory detection of mixed mycobacterial infections can impact patient management and appropriate public health control measures.

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