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

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. intracellularare 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.

2. AFB (+) Culture Workflow
Positive Culture
MGIT broth, 7H11 agar, LJ slant, Blood culture bottle, etc.

Prepare & read Kinyoun Acid Fast Stain

Previous (+) Mycobacteria ID within 2 months

AFB Present

No previous positive culture or (+) Mycobacteria ID >2 months ago

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 I.D. with LPA, reflexed to16S sequencing

Subculture to solid media
- Run LPA
- Visually inspect growth weekly on solid media
- Correlate growth with LPA result

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.

INNO-LiPA MYCOBACTERIA v2 Data Reporting Sheet

4. Total Line Probe Identifications
January 2015 - October 2016

5. Mixed Mycobacterium spp. Detected
January 2015 - October 2016

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. intracellularare (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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