

# Comparison of three methods of *Mycobacterium tuberculosis* complex spoligotype determination for clinical isolates, Lyon, France

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**Declaration of Interests**  
 The authors declare no competing interests.

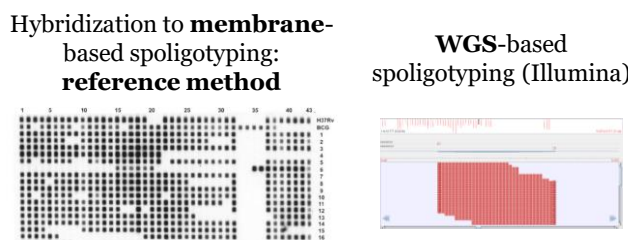
## Background

- To tackle disease spreading : Epidemiological studies to investigate TB transmission chains
- Spacer-oligonucleotide-typing or spoligotyping:
  - Genotyping assay for MTBC clinical isolates
  - Species identification among the MTBC

Here we compared the spoligotype profiles of MTBC clinical isolates by using different methods

## Methods

All MTBC strains isolated from patients during routine practice at the mycobacteria laboratory of Lyon University Hospital, France, between November 2016 and December 2020 were included (n=597) to compare:



Among these MTBC strains, 133 were also analysed by hybridization to microbeads-based spoligotyping: **Beamedex** using Luminex technology

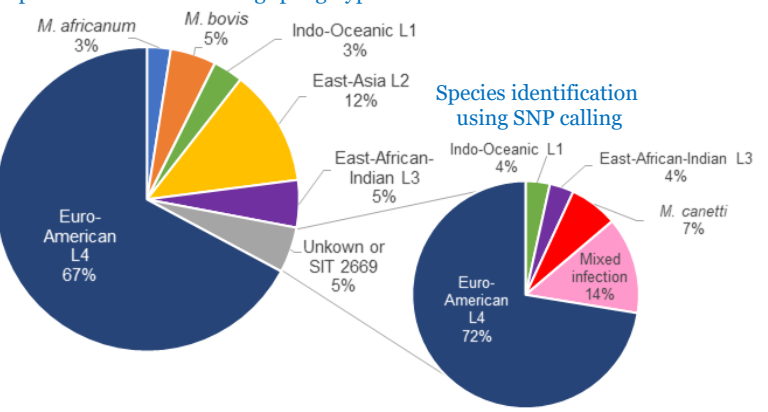


## Results

### Membrane-based vs WGS-based spoligotyping

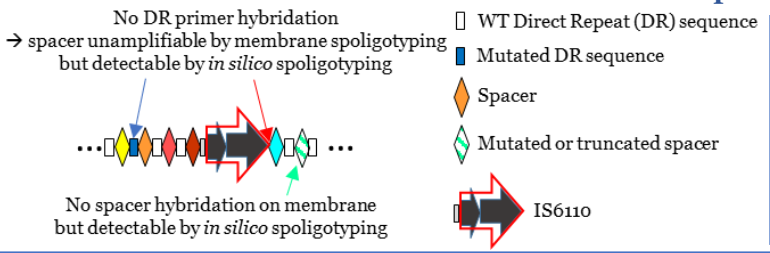
Discrepant spoligotypes	1 spacer	2 spacers	>2 spacers
84/597	74	10	0
14.2%	88.1%	11.9%	0

### Species identification using spoligotype



### CRISPRbuilder-TB:

- DR variants
- Insertion of IS6110 between the spacer and the DR or within the DR
- Mutated or truncated spacers



## Conclusion

- WGS showed very few discrepancies compared to the hybridization-based assay for spoligotyping (including for species identification).
- WGS provided added value in some cases of species identification.

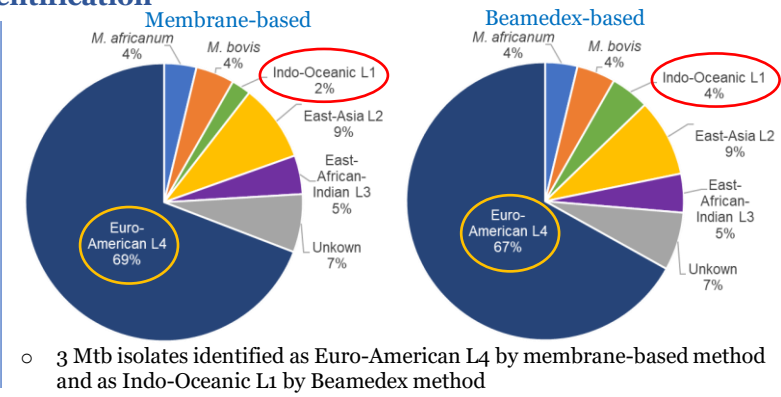
Possibility of a smooth transition from the traditional to the in silico-based genotyping of MTBC isolates upon TB diagnosis and epidemiologic survey

## Overall concordance

### Membrane-based vs Beamedex-based spoligotyping

Discrepant spoligotypes	1 spacer	2 spacers	>2 spacers
28/133	22	3	3
21.4%	78.6%	10.7%	10.7%

## Species identification



- 3 Mtb isolates identified as Euro-American L4 by membrane-based method and as Indo-Oceanic L1 by Beamedex method

## Discrepancy analysis

**Redundant discrepancies:** spacers not detected though membrane-based spoligotyping and detected with Beamedex-based spoligotyping  
 → More sensitive method, higher risks of cross-contaminations

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