

Table SI. Accession numbers of *Mycobacterium* species and subspecies used.

| Species/subspecies identity | Accession numbers | (Refs.) |
|-----------------------------|-------------------------|---------|
| MAB- isolate 1 | SRR14043053 | (19) |
| MAB-isolate 2 | SRR14043043 | |
| MAB-isolate 3 | SRR14043035 | |
| MAB-isolate 4 | SRR14043008 | |
| MAB-isolate 5 | SRR14043007 | |
| MMAS-isolate 1 | SRR14043059 | |
| MMAS-isolate 2 | SRR14043004 | |
| MMAS-isolate 3 | Identified by MALDI-TOF | |
| MMAS-isolate 4 | SRR14043045 | |
| MMAS-isolate 5 | SRR14043031 | |
| MTB-H37Ra (ATCC 25177) | CP000611 | (22) |

The data from these databases are now available in the European Nucleotide Archive. NTM datasets are accessible under BioProject accession PRJNA523980 (<https://www.ebi.ac.uk/ena/browser/view/PRJNA523980>), whereas MTB-H37Ra data is available under PRJNA18883 (<https://www.ebi.ac.uk/ena/browser/view/PRJNA18883>). MAB, *Mycobacterium abscessus* subspecies *abscessus*; MMAS, *Mycobacterium abscessus* subspecies *massiliense*; MTB-H37Ra, *Mycobacterium tuberculosis*-H37Ra; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; NTM, nontuberculous mycobacteria.

Table SII. Specific DNA-related Raman shifts and their corresponding peak assignments.

| Raman shift (cm ⁻¹) | Peak assignments | (Refs.) |
|---------------------------------|---|------------------|
| 726 | Adenine (ring breathing mode) | (26-32) |
| 755 | Thymine (ring breathing mode) | (27,32) |
| 781 | Thymine/Cytosine (ring breathing mode) | (26-28,30-35) |
| 1,097 | Asymmetric stretching mode (O-P-O) ⁻ | (26-28,33) |
| 1,317 | Guanine (C-N stretching mode) | (27,28) |
| 1,331 | Adenine/Guanine | (26-28,32) |
| 1,486 | Guanine/Adenine | (26-28) |
| 1,577 | Guanine/Adenine | (26-28,33,36,37) |

Table SIII. Reproducibility assessment of SERS spectra via Pearson's and Spearman's correlation analysis.

| Isolates | R ² (Repeat 1) | R ² (Repeat 2) | R ² (Repeat 3) | Pearson's r (Mean ± SD) | Spearman's ρ (Mean ± SD) |
|-------------------------|------------------------------|------------------------------|------------------------------|----------------------------|-----------------------------|
| A, At 15 ng/μl (785 nm) | | | | | |
| MAB-isolate 1 | 0.8484 | 0.8402 | 0.8399 | 0.91 ± 0.01 | 0.86±0.01 |
| MAB-isolate 2 | 0.9005 | 0.8830 | 0.8716 | 0.92±0.01 | 0.87±0.01 |
| MAB-isolate 3 | 0.8753 | 0.8669 | 0.8698 | 0.91±0.02 | 0.86±0.02 |
| MAB-isolate 4 | 0.7701 | 0.7651 | 0.7692 | 0.91±0.01 | 0.76±0.01 |
| MAB-isolate 5 | 0.8067 | 0.7947 | 0.8099 | 0.93±0.01 | 0.86±0.01 |
| MMAS-isolate 1 | 0.8408 | 0.8156 | 0.8169 | 0.92±0.01 | 0.86±0.02 |
| MMAS-isolate 2 | 0.8811 | 0.8764 | 0.8745 | 0.92±0.01 | 0.88±0.01 |
| MMAS-isolate 3 | 0.8710 | 0.8711 | 0.8703 | 0.91±0.01 | 0.83±0.02 |
| MMAS-isolate 4 | 0.8176 | 0.8193 | 0.8303 | 0.91±0.00 | 0.80±0.01 |
| MMAS-isolate 5 | 0.8047 | 0.7838 | 0.8217 | 0.88±0.05 | 0.81±0.05 |
| MTB-H37Ra | 0.8144 | 0.7848 | 0.8370 | 0.86±0.11 | 0.81±0.05 |
| B, At 50 ng/μl (785 nm) | | | | | |
| MAB-isolate 1 | 0.8334 | 0.8217 | 0.8074 | 0.90±0.02 | 0.84±0.03 |
| MAB-isolate 2 | 0.8891 | 0.8853 | 0.8709 | 0.94±0.02 | 0.87±0.04 |
| MAB-isolate 3 | 0.8645 | 0.8561 | 0.8555 | 0.92±0.01 | 0.84±0.01 |
| MAB-isolate 4 | 0.8289 | 0.8552 | 0.8503 | 0.94±0.00 | 0.84±0.01 |
| MAB-isolate 5 | 0.8282 | 0.8634 | 0.8526 | 0.92±0.02 | 0.79±0.02 |
| MMAS-isolate 1 | 0.9045 | 0.8998 | 0.8967 | 0.92±0.01 | 0.83±0.03 |
| MMAS-isolate 2 | 0.9014 | 0.9005 | 0.9020 | 0.92±0.01 | 0.82±0.02 |
| MMAS-isolate 3 | 0.8633 | 0.8448 | 0.8792 | 0.90±0.04 | 0.80±0.02 |
| MMAS-isolate 4 | 0.7910 | 0.8177 | 0.8451 | 0.89±0.05 | 0.82±0.04 |
| MMAS-isolate 5 | 0.8813 | 0.8563 | 0.8659 | 0.91±0.04 | 0.85±0.05 |
| MTB-H37Ra | 0.9170 | 0.9160 | 0.9143 | 0.92±0.02 | 0.83±0.02 |

MAB, *Mycobacterium abscessus* subspecies *abscessus*; MMAS, *Mycobacterium abscessus* subspecies *massiliense*; MTB-H37Ra, *Mycobacterium tuberculosis*-H37Ra; R², the coefficient of determination; SD, standard deviation.