CLAIMS

1. A method of identifying a mycobacterium using surface enhanced Raman spectroscopy (SERS), the method comprising:

disposing a sample suspected to comprise a mycobacterium on a SERS-active substrate;

detecting surface enhanced Raman signals corresponding to an alpha-mycolic acid, a methoxy-mycolic acid, and a keto-mycolic acid from the sample disposed on the SERS-active substrate; and

determining one or more ratios of intensity of the surface enhanced Raman signals to identify the mycobacterium,

wherein determining the one or more ratios of intensity of the surface enhanced Raman signals comprises:

correlating the intensity of the surface enhanced Raman signals corresponding to the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid to a concentration for each of the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid detected in the sample; and

comparing the concentrations to obtain the one or more ratios for identifying the mycobacterium.

2. The method of claim 1, wherein disposing the sample comprises providing the sample in the form of a liquid.

3. The method of claim 2, wherein providing the sample in the form of a liquid comprises mixing the sample in a solvent.

4. The method of claim 3, wherein the solvent comprises chloroform and methanol.

5. The method of claim 4, wherein the chloroform and methanol are present in a volume ratio of 1:1 to 9:1.

6. The method of any one of claims 1 to 5, wherein the sample suspected to comprise the mycobacterium is not subjected to an extraction process to extract the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid from the mycobacterium suspected to be comprised in the sample.

7. The method of any one of claims 1 to 5, further comprising extracting the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid from the mycobacterium suspected to be comprised in the sample prior to disposing the sample on the SERS-active substrate.

8. The method of claim 7, wherein extracting the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid comprises disintegrating a cell wall of the mycobacterium suspected to be comprised in the sample.

9. The method of claim 7 or 8, wherein extracting the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid comprises subjecting the sample to saponification, mechanical milling, delipidating, or a combination thereof.

10. The method of any one of claims 7 to 9, further comprising removing lipids from the sample prior to disposing the sample on the SERS-active substrate.

11. The method of any one of claims 7 to 10, wherein extracting the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid comprises delipidating the mycobacterium suspected to be comprised in the sample.

12. The method of claim 11, wherein the delipidating comprises:
inactivating the mycobacterium suspected to be comprised in the sample; and
separating the sample into an aqueous phase and an organic phase.

13. The method of claim 12, wherein inactivating the mycobacterium comprises incubating the sample at a temperature ranging from 20°C to 40°C.

14. The method of claim 13, wherein incubating the sample is carried out for at least 24 hours.

15. The method of any one of claims 12 to 14, further comprising:

mixing the aqueous phase with one or more organic solvents to form a mixture;

separating the mixture into a further aqueous phase, a further organic phase and an intermediate phase, wherein the intermediate phase comprises the delipidated mycobacterium suspected to be comprised in the sample.

16. The method of claim 15, wherein the one or more organic solvents comprise chloroform.

17. The method of claim 15 or 16, further comprising drying the intermediate phase to obtain the delipidated mycobacterium suspected to be comprised in the sample.

18. The method of claim 17, further comprising:

contacting the delipidated mycobacterium suspected to be comprised in the sample with a mixture of alcohol and a base;

heating the resultant mixture; and

adding an extraction solvent and an acid to the heated mixture.

19. The method of claim 18, wherein the extraction solvent comprises chloroform.

20. The method of claim 18 or 19, wherein the acid comprises hydrochloric acid.

21. The method of anyone of claims 1 to 20, wherein disposing the sample on the SERS-active substrate comprises mixing the sample in a solvent before disposing the sample on the SERS-active substrate.

22. The method of any one of claims 1 to 21, wherein the SERS-active substrate comprises or consists of gold, silver, or an alloy thereof.

23. The method of claim 22, wherein the SERS-active substrate comprises nanoparticles of gold, silver, an alloy thereof, or a mixture thereof.

24. The method of claim 22, wherein the SERS-active substrate is a support comprising at least one SERS-active nanostructure disposed on a surface of the support.

25. The method of claim 24, wherein the support comprising at least one SERS-active nanostructure disposed on a surface of the support is one of a dielectric support, a semiconductor support, or a paper support, the support comprising at least one nanostructure coated with a layer of a SERS-active material.

26. The method of claim 25, wherein the SERS-active material comprises a layer of gold, silver, or an alloy thereof.

27. The method of claim 26, wherein the support comprises a silicon substrate having an array of nanostructures disposed thereon.

28. The method of claim 27, wherein the nanostructures comprise silicon nanopillars.

29. The method of any one of claims 1 to 28, wherein detecting the surface enhanced Raman signals comprises exposing the sample disposed on the SERS-active substrate to an electromagnetic radiation and generating one or more surface enhanced Raman signals from the sample.

30. The method of claim 29, wherein the electromagnetic radiation has a wavelength ranging from 100 nm to 1000 nm.

31. The method of claim 30, wherein the electromagnetic radiation has a wavelength of 785 nm.

32. The method of any one of claims 29 to 31, wherein the one or more surface enhanced Raman signals generated are in the range of 600 cm^{-1} to 1800 cm^{-1}.

33. The method of any one of claims 29 to 32, wherein detecting the surface enhanced Raman signals comprises comparing the one or more surface enhanced Raman signals generated from the sample with the surface enhanced Raman signals generated from an artificially synthesized alpha-mycolic acid, an artificially synthesized methoxy-mycolic acid, and an artificially synthesized keto-mycolic acid.

34. The method of any one of claims 1 to 33, wherein detecting the surface enhanced Raman signals comprises determining presence of and/or intensity of surface enhanced Raman signals corresponding to the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid.

~~35. The method of any one of claims 1 to 34, wherein determining the one or more ratios of intensity of the surface enhanced Raman signals comprises:~~

~~correlating the intensity of the surface enhanced Raman signals corresponding to the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid to a concentration for each of the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid detected in the sample; and~~

~~comparing the concentrations to obtain the one or more ratios for identifying the mycobacterium.~~

~~36~~35. The method of any one of claims 1 to ~~35~~34, wherein the mycobacterium is a non-tuberculous mycobacterium or a species of mycobacterium tuberculosis complex.

~~37~~36. The method of claim ~~36~~35, wherein the non-tuberculous mycobacterium comprises *mycobacterium avium* complex, *mycobacterium kansasii*, or *mycobacterium abscessus*.

~~38~~37. The method of claim ~~36~~35 or ~~37~~36, wherein the species of mycobacterium tuberculosis complex comprises *mycobacterium tuberculosis*, *mycobacterium africanum*, *mycobacterium bovis*, or *mycobacterium microti*.

~~39~~38. The method of any one of claims 1 to ~~38~~37, wherein the sample suspected to comprise mycobacterium contains any of the alpha-mycolic acid, the methoxy-mycolic acid, and the keto-mycolic acid present in a concentration ranging from 1 nM to 1 μM.

~~40~~39. The method of any one of claims 1 to ~~39~~38, wherein the method is carried out *in vitro* or *ex vivo*.

~~41~~40. The method of any one of claims 1 to ~~40~~39, wherein the method is used for detecting a mycobacterial disease.

~~42~~41. The method of claim ~~41~~40, wherein the mycobacterial disease comprises a pulmonary disease, a lymphatic disease, or a skin disease.

~~43~~42. The method of claim ~~41~~40 or ~~42~~41, wherein the mycobacterial disease comprises tuberculosis.

~~44~~43. A method of detecting a mycobacterial disease using surface enhanced Raman spectroscopy (SERS), the method comprising:

identifying a mycobacterium according to the method of any one of claims 1 to ~~43~~42; and

determining the mycobacterial disease based on the mycobacterium identified.

~~45~~44. The method of claim ~~44~~43, wherein the mycobacterial disease comprises a pulmonary disease, a lymphatic disease, or a skin disease.

~~46~~45. The method of claim ~~44~~43 or ~~45~~44, wherein the mycobacterial disease comprises tuberculosis.

~~47~~46. The method of any one of claims ~~44~~43 to ~~46~~45, wherein the method is carried out in vitro or ex vivo.