Technical Note 104
Preparing Mycobacteria Sample Extracts

The Sherlock Mycobacteria Identification System analyzes the composition of mycolic acids found in the cell wall to identify mycobacterial species or groups. To release the compounds from the cell wall and to make the samples safe to handle, the harvested cells are saponified in an autoclave. Because the mycobacteria are killed by the saponification procedure, the procedure is safe from a microbiology standpoint from the first step forward.

The mycolic-acids are converted to free acids by lowering the pH, extracted into chloroform to remove materials not soluble in organic solvents and derivatized by a fluorescent derivatizing agent.

Culture Growth

The mycobacteria cultures typically are grown on solid medium such as Middlebrook 7H10 (or 7H11) at 35-37C, until visible growth is noted. Some species of mycobacteria may normally be grown under different conditions and the database has been constructed to reflect this. Examples would be the culturing of *M. marinum* and *M. haemophilum* at 30C, with the latter organism being grown on chocolate agar rather than on Middlebrook . Examination of cultures under a dissecting microscope may aid in detection of potentially mixed cultures of the slow-growing organisms.

Sample Preparation

There are three steps required to prepare samples to be run on the HPLC, and these are outlined in the following sections.

I. Harvesting Cultures and Saponification

1. Obtain a sufficient number of 13 x 100 mm screw cap tubes for the sample batch. Each batch should contain a known strain as a positive control and a negative control. For a positive control use *M. gordonae* ATCC 14470. For a negative control use *Candida albicans* ATCC 60193. Carefully inspect each tube for defects and proper cap fit. Discard any defective tubes/caps.

2. Label tubes with appropriate identifier using a laboratory-marking pen that will withstand autoclave conditions.

3. Add 1.0ml of Saponification Reagent **(Reagent 1)** to each tube using a repeat dispenser.

4. Harvest growth from the agar plate into the tube containing the saponification reagent. Only a very small amount of cells is needed therefore an amount of cells barely visible on the end of a

sterile applicator stick or disposable plastic bacteriological loop is sufficient. It is better to work with isolated colonies.

5. Securely tighten a rubber-lined cap onto each tube.

6. Vortex each tube for 5-10 seconds.

7. Place autoclave indicator tape on the rack containing the tubes.

8. Autoclave for 30-60 minutes at 121°C with slow exhaust.

II. Extraction

1. Remove tubes from autoclave and allow them to cool to ambient temperature.

2. Add 1.8ml of Acidification Reagent **(Reagent 2)** with the repeat dispenser. Cap the tubes and invert as necessary **(2-3x)** to obtain thorough mixing. Allow the tubes to stand for 5 minutes.

3. Uncap tubes and add 1.5ml of chloroform **(Reagent 3)** using the repeat dispenser.

4. Recap each tube and vortex vigorously for 30-60 sec. Visually inspect each tube to insure full vortex.

5. After phase separation, transfer the bottom (chloroform) layer to a clean 13 x 100 mm borosilicate glass tube. Note: It may be necessary to briefly centrifuge the tube if an emulsion is present. The tube must be labeled with the corresponding label number from the original 13 x 100mm tube.

6. Evaporate the chloroform from each tube.

7. Cool the tubes to ambient temperature.

III. Derivatization

1. Add 200ul Derivatizing Reagent **(Reagent 4)** with dispenser.

2. Gently swirl the tube to dissolve its contents.

3. Transfer the mixture from each tube into a correspondingly labeled amber vial (2 ml size), which has had the interior precoated with 100ul of 2% Methanolic Potassium Bicarbonate **(Reagent 5)**.

4. Swirl the vial gently by hand to assure mixing of the contents.

5. Heat the vials uncapped for 10 minutes at 60±2°C.

6. Slowly evaporate the contents of each vial.

7. Cool to ambient temperature.

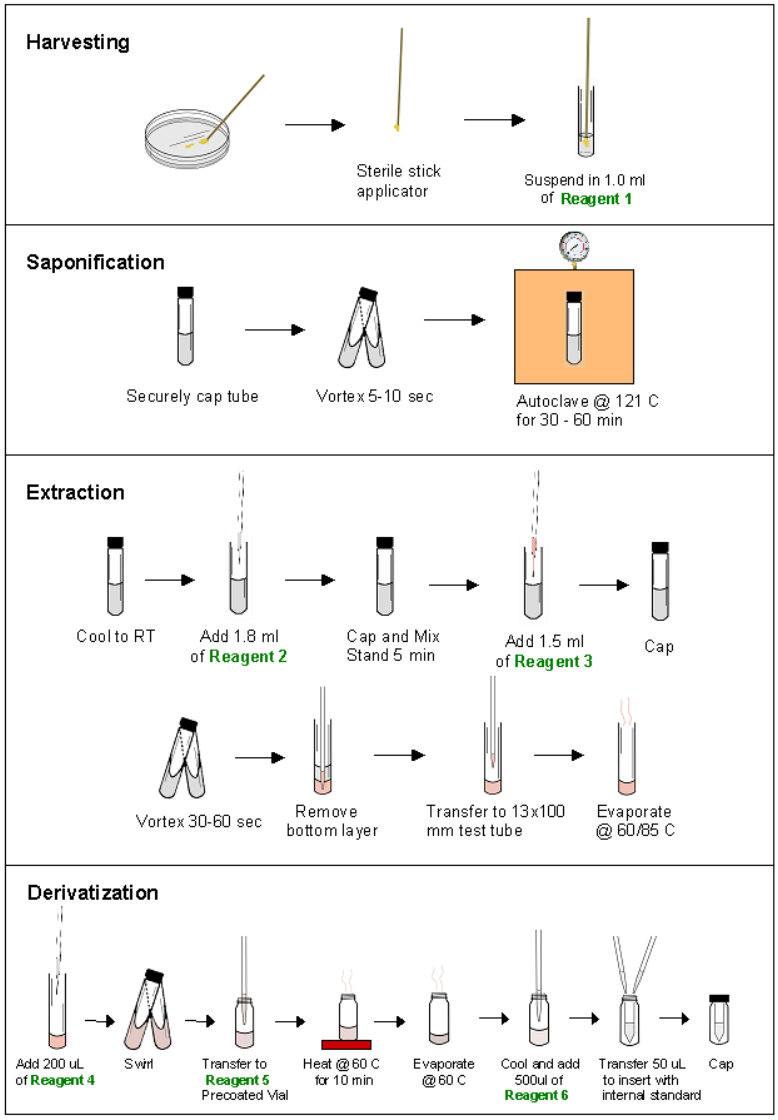
8. Add 500uL of isopropyl alcohol (IPA) **[Reagent 6]** and cap the vial.

9. Swirl to totally dissolve the contents and mix the sample extract.

10. To a tapered insert vial containing the internal standards (MIDI Part # 1600-A) add 50 ul of the sample extract.

11. Once dispensed, thoroughly mix by slowly pumping the solution up and down in the pipette.

12. After capping the vials, the samples are ready for HPLC analysis**.**



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References:

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