

Sample preparation for in vitro mycobacterial Profiling (In vitro Core – SBRI:Seattle Biomedical Research Institute. revised March 31, 2010)

1. At baseline and each planned time point:
2. Harvest 180 mL of culture in 6 x 30 mL aliquots in 50 mL polypropylene (PP) tubes. Centrifuge for 5 minutes at 5000 g, 4°C.
3. At each time point decant supernatant from all 6 samples for proteomics and metabolomics into a 250 mL Steriflip filter sterilizer, pull through the 0.2 micron filter using vacuum, and then repeat in a second Steriflip.
4. A 10 mL aliquot of each sterilized supernatant will be set aside for metabolomics and stored at -80°C. The remaining supernatant will be transferred to 50 mL conical stored at -80°C until the experiment is complete and then sent on for proteomics analysis.
5. Pellets are stored at -80°C until the time course is complete.
6. Divide the pellets into six sets comprising a single pellet from each time point. These pellet sets will be used for:
 - a. Transcriptomics
 - b. Two sets for Proteomics/Glycomics
 - c. Lipidomics
 - d. Metabolomics
 - e. Reserver (banked at -80°C at SBRI)
7. The pellet set for transcriptomics will be processed into RNA using a standard GTC+bead beating protocol at SBRI.
8. The reserve pellet set will be banked at SBRI until in case problems arise with any of the other samples sets.
9. Wash the remaining four sets of pellets in 10 mL 1X PBS, room temp, centrifuge for 5 minutes at 5000 g, 4°C.
10. Resuspend all four pellets from each time point in 40 mL PBS and then redivide into 10 mL aliquots: two aliquots for proteomics, one for lipidomics and one for metabolomics.
11. Label products as “MTB log phase” or “MTB Hypoxemia”, the name of the platform, the time point and the date.
12. Centrifuge and discard PBS as in step 4.

Sample preparation for lipidomics and metabolomics

1. Re-suspend pellet (which must be 0.5 ml in volume or less) in 10 mL Chloroform:Methanol (C:M) (2:1, V:V), transfer to a 15 mL glass tube, cap tightly and vortex for one minute to distribute solvent. Let it sit for 1 hr at room temperature to sterilize.
2. Place tube on a rotating platform at 37°C, and extract for an additional 1 hr at room temperature. Treat outside of tube with chemical disinfectant and remove from BSL-3.
3. Evaporate to dryness under nitrogen. Store samples at -80°C until ready to ship.
4. Check sterility of one control pellet of C:M treated cells as outlined in **Note 1**. Once samples are confirmed sterile, ship to Metabolon and BWH on dry ice as non-infectious

material.

Sample preparation for proteomics

13. Re-suspend the pellet in 1.5 ml 6M guanidinium thiocyanate in a 2 ml screw cap tube.
14. Incubate for 2 hours in a rotator at 37°C.
15. Transfer sterilized pellets to a -80°C freezer until shipping to PPD.
16. Check sterility of one control pellet of GTC treated cells as in Note 1 and ship to PPD on dry ice as non-infectious material.

Note 1.

Pellet the GTC and discard the supernatant. Add to both the GTC sterilized pellet and the dried C:M treated extract 100 ul of 7H9 media. Plate 100 ul suspension on 7H10 or 7H11 plates (10 x). Allow solvent to evaporate off plates. Incubate plates at 37°C for 21 days and check for Mtb growth. If no colonies are visible at 21 days, the samples are considered sterile. Maintain the plates to 60 days and check for any visible growth.