

**SOP: PP017**

**SOP for QC Analysis of LAM and LM**

**Materials and Reagents:**

1. Pure LAM or LM
2. Endotoxin Free Water
3. D<sub>2</sub>O, 99%
4. D<sub>2</sub>O, 100%, 0.75ml vials
5. Alditol Acetate Reagents
6. SDS-PAGE Supplies
7. Western Blotting Supplies
8. CS-35,  $\alpha$ -LAM Antibody
9. LAL Assay Reagents
10. 16x100mm Glass Culture Tubes with Screw Caps
11. 13x100mm Glass Culture Tubes with Screw Caps
12. NMR Tube
13. 1.2ml Cryovials
14. Savant
15. GC

**Protocol:**

1. \_\_\_\_\_ Resuspend dried sample in endotoxin free water at a concentration of about 10mg/ml based on weight and transfer to 16x100 glass tube.
2. \_\_\_\_\_ Remove approximately half of the sample and transfer to another tube (note 1) and dry on savant (note 2).
3. \_\_\_\_\_ To the dried material, add 1ml 99% D<sub>2</sub>O and dry.
4. \_\_\_\_\_ Repeat D<sub>2</sub>O exchange (step 3) once more.
5. \_\_\_\_\_ Add the entire contents of one vial of 100% D<sub>2</sub>O. Get sample into suspension and then transfer to a clean NMR tube.
6. \_\_\_\_\_ Run NMR to check for contaminants from buffers (note 3).
7. \_\_\_\_\_ If NMR is clean, transfer sample back into the 16x100 glass tube and place on the savant to dry.
8. \_\_\_\_\_ Resuspend sample in the same volume of water as was removed in step 2. This will restore the sample to its original concentration.
9. \_\_\_\_\_ From the original sample (the half not being used for NMR), transfer 50 $\mu$ g aliquots to each of three 13x100 glass tubes.
10. \_\_\_\_\_ Perform alditol acetate derivation on sample (note 4).
11. \_\_\_\_\_ Run GC on sample and calculate the concentration of LAM or LM (note 5).
12. \_\_\_\_\_ Based on the calculated concentration from step 11, run 3 $\mu$ g sample on a gel and 10 $\mu$ g of sample on a western blot (note 6).
13. \_\_\_\_\_ Place a 100 $\mu$ l aliquot of sample in a cryovial for LAL analysis.
14. \_\_\_\_\_ Run LAL assay in triplicate and calculate endotoxin amount relative to your sample concentration (note 7).

15. \_\_\_\_\_ Aliquot samples into 0.25mg, 0.5mg, and 1mg aliquots in cryovials. Dry on the savant, label and store at -80 °C.

**Notes:**

1. Be sure to record the exact volume removed because this is the volume that will be added back to the sample after the NMR is complete. While D2O exchanges and NMR are being performed on this half of the sample, you can continue the QC with the rest of the sample, starting with step 9.
2. See SOP SP005 for operation of the Savant
3. If there are contaminants, repeat the dialysis described in steps 16-24 in the SOP PP016 for separation of LAM, LM, and PIM
4. See SOP SP022 for preparation of Alditol Acetate Derivatives
5. To calculate the concentration of LAM or LM in the sample, add the µg quantities for Mannose and Arabinose given by the GC analysis. Divide this number by the volume of the aliquot used to prepare the alditol acetate. Then multiply by 1.25 (this is a conversion factor). This gives the concentration. Use the average of all three GC runs as your final concentration. For determination of M. smeg LAM use the correction factor of 1.11 instead of 1.25  
$$((\mu\text{g Arabinose} + \mu\text{g Mannose}) / \mu\text{l Sample}) \times 1.25 = \text{concentration}$$
6. See SOP SP007 for running SDS-PAGE gels, SOP SP012 for Silver Staining (use periodic acid step), and SOP SP011 for Western Blot. When developing the western blot, use CS-35 as the primary antibody and anti-mouse IgG as the secondary antibody
7. See SOP SP020 for LAL Assay. To calculate endotoxin amount, take the concentration given by the analysis (endotoxin units per ml) and divide by 10 (conversion factor to give you ng of endotoxin), then divide by the concentration of sample. This will give you ng endotoxin/mg sample. The endotoxin amount should be less than 10ng/mg
8. Make more of the 0.5mg aliquot because this is the default quantity given to investigators