SOP: SP079
Created: 09/19/2022 by MS

Quantitation of LAM by Image J

Materials and Reagents:

1. Purified lipoarabinomannan (see SOP PP015-PP017 for LAM, or PP059 for Demannosylated LAM)
2. QC qualified *M. smegmatis* lipoarabinomannan of known concentration
3. SDS-PAGE Gel supplies
4. Silver staining supplies
5. Computer with scanning capabilities

Protocol:

1._____ Using a smegLAM standard with a known concentration, create a standard curve on the gel ranging from 1µg - 5µg in 0.5ug increments. Run the unknown sample in various volumes (typically 0.5µl – 2µl)

2._____ Run sample and standard curve on a gel, and stain by silver stain using periodic acid (note 1)

3._____ Scan the stained gel to obtain a clear and useable image.

4._____ Open Image J software (note 2). Using the toolbar, enter the 'File" dropdown menu and select "Open" to find your image.

5._____ Beginning with your most concentrated standard, click and drag the computer mouse to create a box around this band. Be sure to capture the entire band, including the faint portions.

6._____ With the band highlighted, go to the tool bar and find the "Analyze" dropdown menu. Select "Measure". This should prompt a table to appear with your data.

7._____ Drag the **same sized box** (by selecting in the middle of it) to the next standard (note 3). Repeat step 6. This should add to your results table. Continue this process until you have recorded data for all your standards and samples.

8._____ Copy data from the table in Image J, then paste it into an Excel spreadsheet

9._____ Insert your standard amounts and sample volumes into the spreadsheet (note 4).

10._____ Plot the standard amount vs the "Mean Gray Scale" data (x-axis = quantity; y-axis = mean gray scale) (note 5).

11._____ Using the line of best fit equation and the mean gray scale value of each sample band, solve for the concentration of each sample loaded onto the gel (notes 6 & 7).

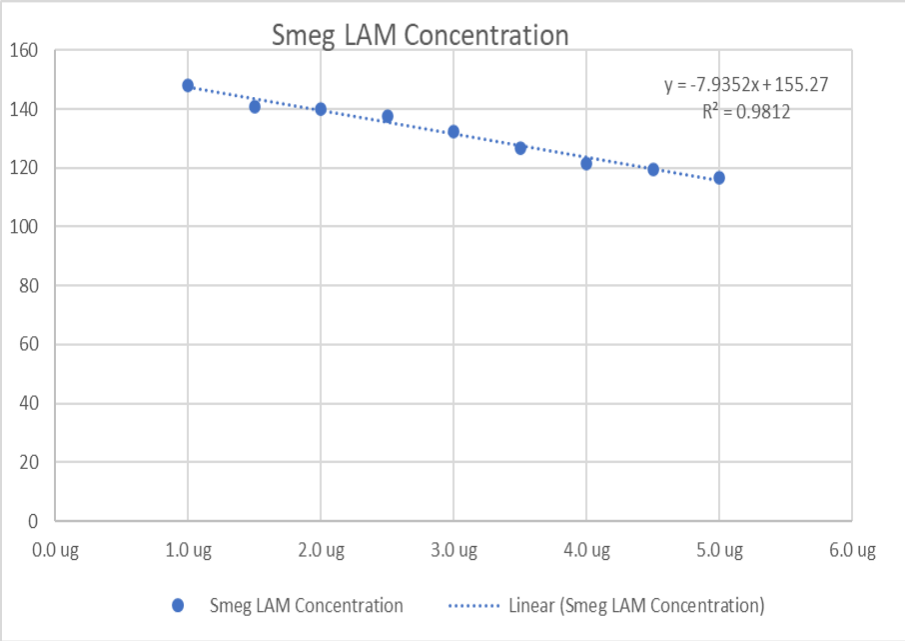
Notes:

1. See SOP SP007 for running SDS-PAGE gels and SOP SP012 for Silver Staining (use periodic acid step).
2. If this program is not downloaded on the computer, go to https://imagej.nih.gov/ij/ . From the homepage you can download the program, or select "Run Image J in browser".
3. The box will now likely surpass the band and include some white space – this is to be expected and is important for the densitometry calculations. Do not alter the box size.
4. The table should follow the format below. Columns A-E should be filled in at this point (Sample values indicated with purple; standards are black):

(A) Amount	(B) Area	(C) Mean Gray Scale	(D) Min	(E) Max	(F) Concentration
5.0 µg	15453	116.795	57	153	
4.5 µg	15453	119.394	57	153	
4.0 µg	15453	121.445	61	154	

3.5 µg	15453	126.782	65	157	
3.0 µg	15453	132.19	75	160	
2.5 µg	15453	137.547	83	163	
2.0 µg	15453	139.879	93	166	
1.5 µg	15453	140.975	96	169	
1.0 µg	15453	148.215	118	172	
2.0 µl	15453	141.24	108	184	((C14-155.27)/-7.9352)/A14= 0.884036
3.0 µl	15453	132.419	78	181	((C13-155.27)/-7.9352)/A13= 0.9599
4.0 µl	15453	137.124	91	178	((C12-155.27)/-7.9352)/A12= 0.571693
5.0 µl	15453	136.988	91	175	((C11-155.27)/-7.9352)/A11= 0.460782

5. Standard Curve using example data above (if not using a template, use excel to create a scatter plot similar to the one below):



6. Complete column F (concentration) of the table using a formula derived from algebra. For sample data above: concentration = ((mean gray scale-155.27)/-7.9352)/amount in µl.
7. Use an average of the calculated concentrations for all sample volumes, excluding those with a gray scale value outside the standard curve. Final concentration for this data would be: (0.884036+0.9599+0.57169+0.460782)/4 = 0.7191 mgs/ml. If all values fall outside the curve, it will be necessary to re-run the gel using different sample volumes.