Overview
- Fatty acid profiles of bacteria were analyzed using CaO as a matrix replacement, which has been shown to cleave fatty acids in situ from lipids during ionization in a MALDI MS instrument.
- Principle components analysis and cross-validation of bacterial fatty acid spectra showed distinct groupings and good correlation.
- MOLI MS could provide clinicians with a fast, reliable method for bacterial ID using the same instrumentation increasingly used for protein-based bacterial diagnostic identification.

Introduction
- Early clinical bacterial identification is crucial for effective treatment.
- Bacterial ID based on fatty acid (FA) profiles was previously reported.¹
- Metal oxide laser ionization mass spectrometry (MOLI MS) is a new technique using CaO as a matrix replacement to cleave lipids and effectively ionize a sample without producing interfering peaks in the low mass region of the mass spectrum.²
- This extends the usable range of matrix-assisted laser desorption/ionization (MALDI) to small molecules.
- FA profiles of ten bacterial species were analyzed by MOLI MS and principal components analysis (PCA) to provide a 3-D visualization of the data.
- Cross-validation was used for bacterial ID and model correlation.

Methods
- Bacteria were cultured overnight on either brain-heart infusion or Lauria Bertani media.
- Individual colonies were suspended in 200 μL of phosphate buffered saline, pH 7.4 (PBS).
- Lipids were extracted by addition of 200 μL of 3:1 hexane/MeOH.
- 100 mg of CaO was mixed with 1 mL of hexane; 1 μL of the resulting slurry was spotted on a MALDI plate.
- One μL of 0.15M HCl in methanol was added to activate CaO.
- Measurements were taken on a JEOL IMS-S3000 SpiralTOF mass spectrometer.

Results
- NMOF analysis of the positive-ion data produced one false positive, but only 4 false negatives out of 470 measurements.
- Cross-validation of the negative-ion data resulted in one false positive and 15 false negatives out of 470 measurements.

Conclusions
- MOLI MS combined with PCA for analysis of FA peaks in bacteria showed distinct groupings for each species. Negative-ion mode showed much better separation of species. Both positive- and negative-ion modes had excellent cross-validation records, demonstrating the effectiveness of the model. MOLI MS can provide clinicians a rapid, reproducible, and cost-effective bacterial ID diagnostic tool.

References

Figure 1: MOLI MS spectra of E. coli fatty acids in positive-ion (a) and negative-ion (b) modes

Figure 2: PCA plot of fatty acid spectra collected in positive-ion mode.

Figure 3: PCA plot of fatty acid spectra collected in negative-ion mode.