

Identification of clinically relevant microbes directly from culture with the MasSpec Pen

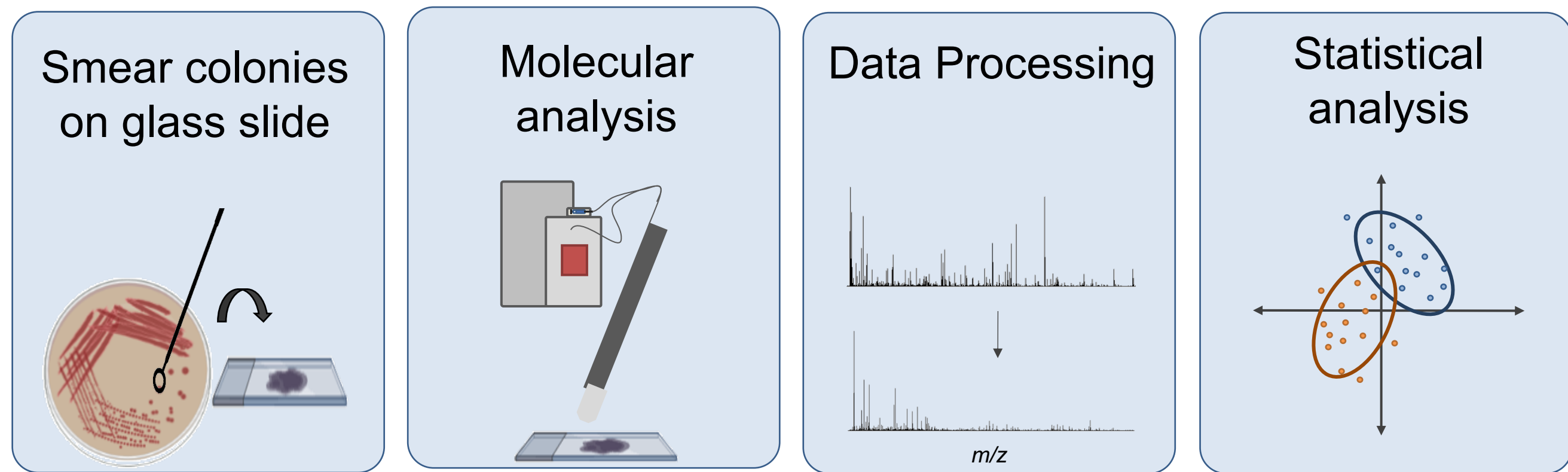
OVERVIEW

- The MasSpec Pen is a handheld mass spectrometry-based device that has been applied for rapid analysis and classification of *ex vivo* tissue.^{1,2}
- Here, the MasSpec Pen was used to directly analyze biomolecules from bacterial culture isolates.
- A lasso statistical classifier was developed to classify bacteria by Gram type, genus, and species with accuracies of 83-100%

INTRODUCTION

- Rapid identification of bacterial pathogens is critical for initiating effective therapy and employing sound antimicrobial stewardship practices.
- The MasSpec Pen has been employed for direct analysis, molecular profiling, and classification of *ex vivo* human tissue with accuracy >96%.^{1,2}
- Here, we employ the MasSpec Pen to identify clinically relevant microbes directly from culture isolates.

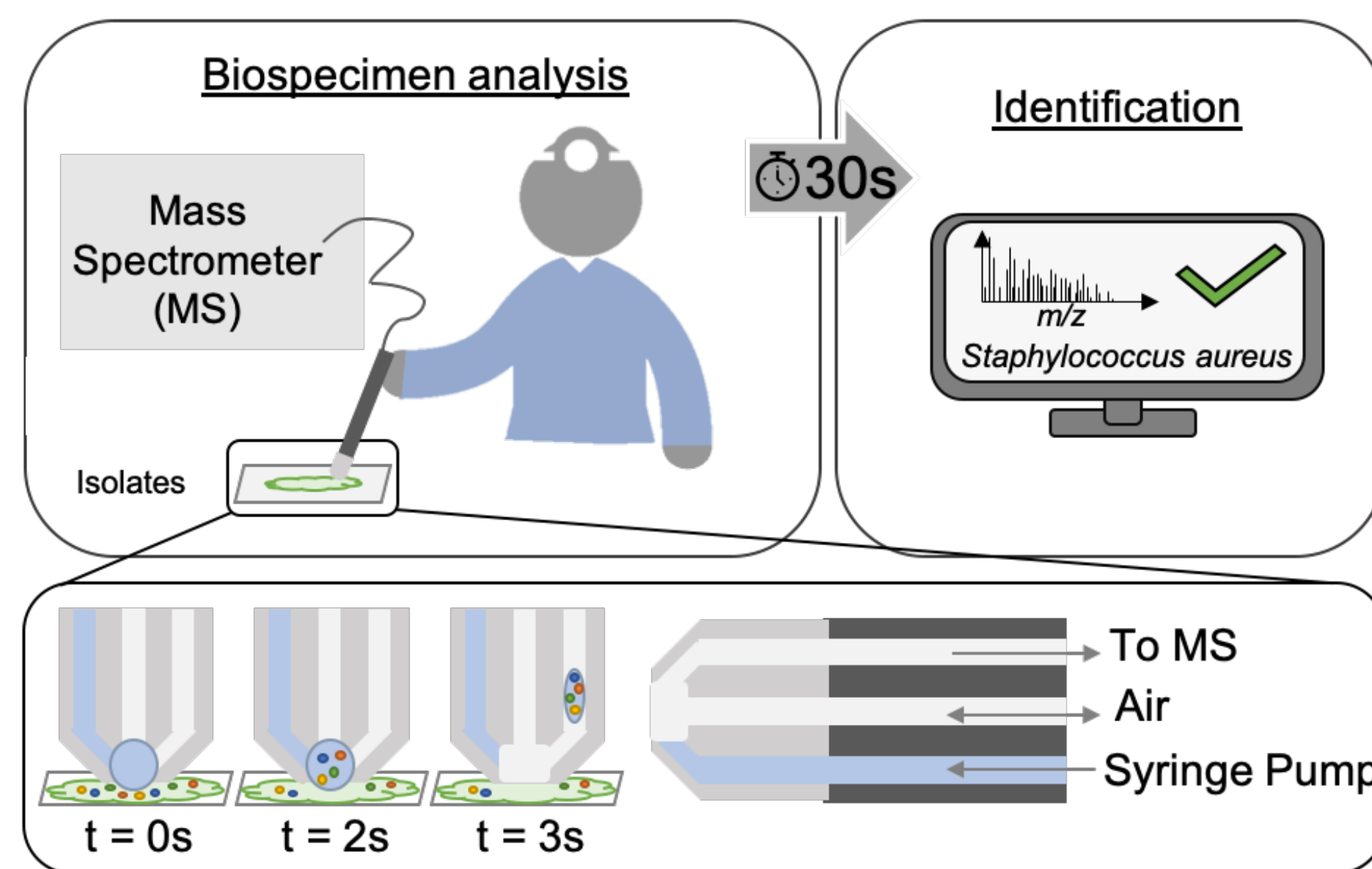
METHODS



Sample Preparation

- Bacteria were cultured at 37 °C overnight on 5% sheep's blood agar.
- Colonies were removed from the agar plate and smeared on a glass slide for analysis.

MasSpec Pen Analysis



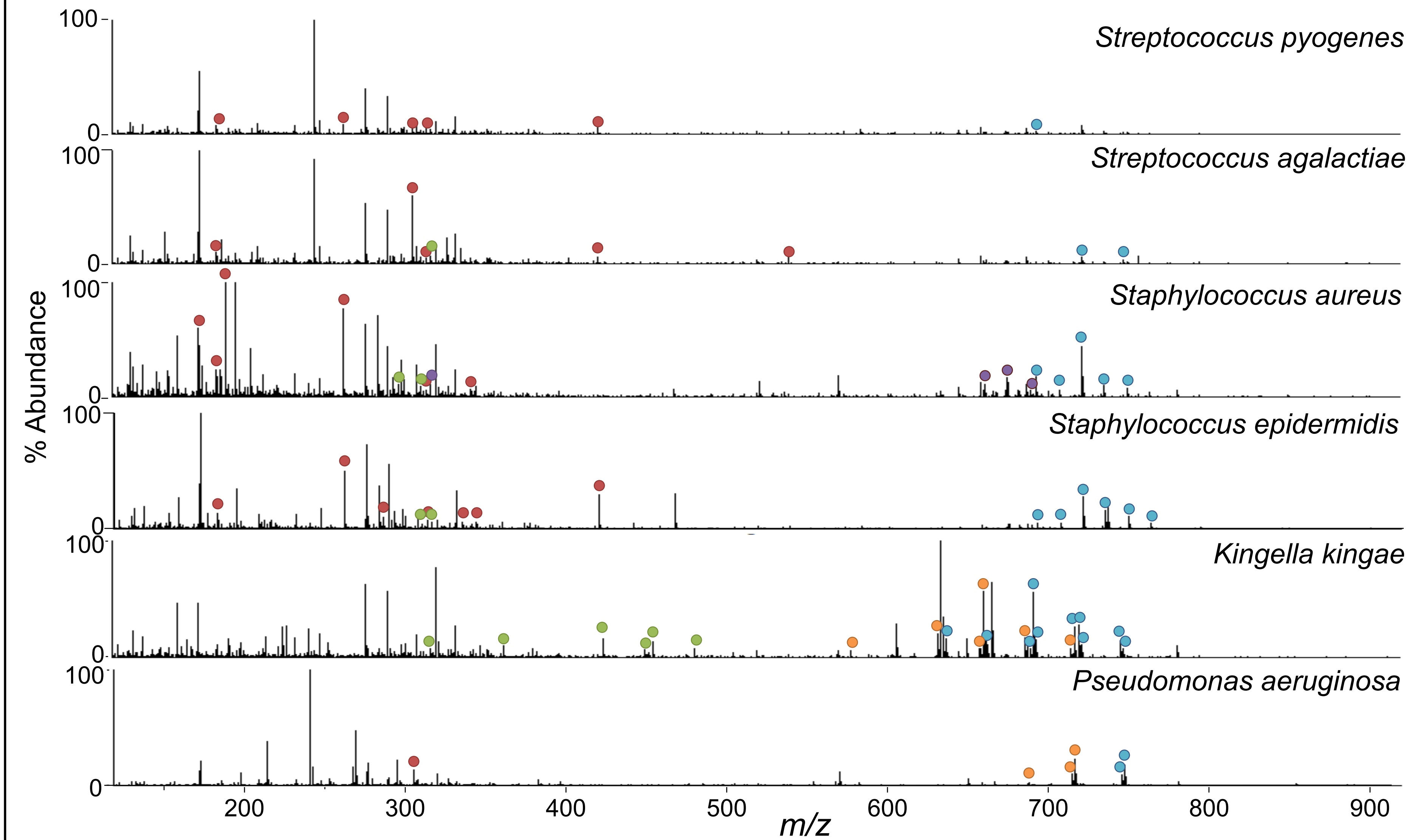
- A 2.7 mm pen tip was applied to the bacterial smear where a 10 µL drop of water was deposited and biomolecules were extracted. After 3 seconds, the droplet was aspirated to the mass spectrometer for analysis.
- Mass spectra were acquired from *m/z* 100-1200 using a Q-Exactive HF Thermo Orbitrap mass spectrometer with a resolving power of 120,000.

Data Processing and Statistical Analysis

- Mass spectra were binned to 0.01 *m/z*. *M/z* features resulting from the agar medium and analysis solvent were removed and data was normalized to the total ion current prior to statistical analysis.
- The least absolute shrinkage and selection operator (lasso)³ was used to select a sparse set of molecular features that are predictive of Gram type, genus, and species. Leave-one-out cross validation and a validation set of samples were used to evaluate model performance.

RESULTS

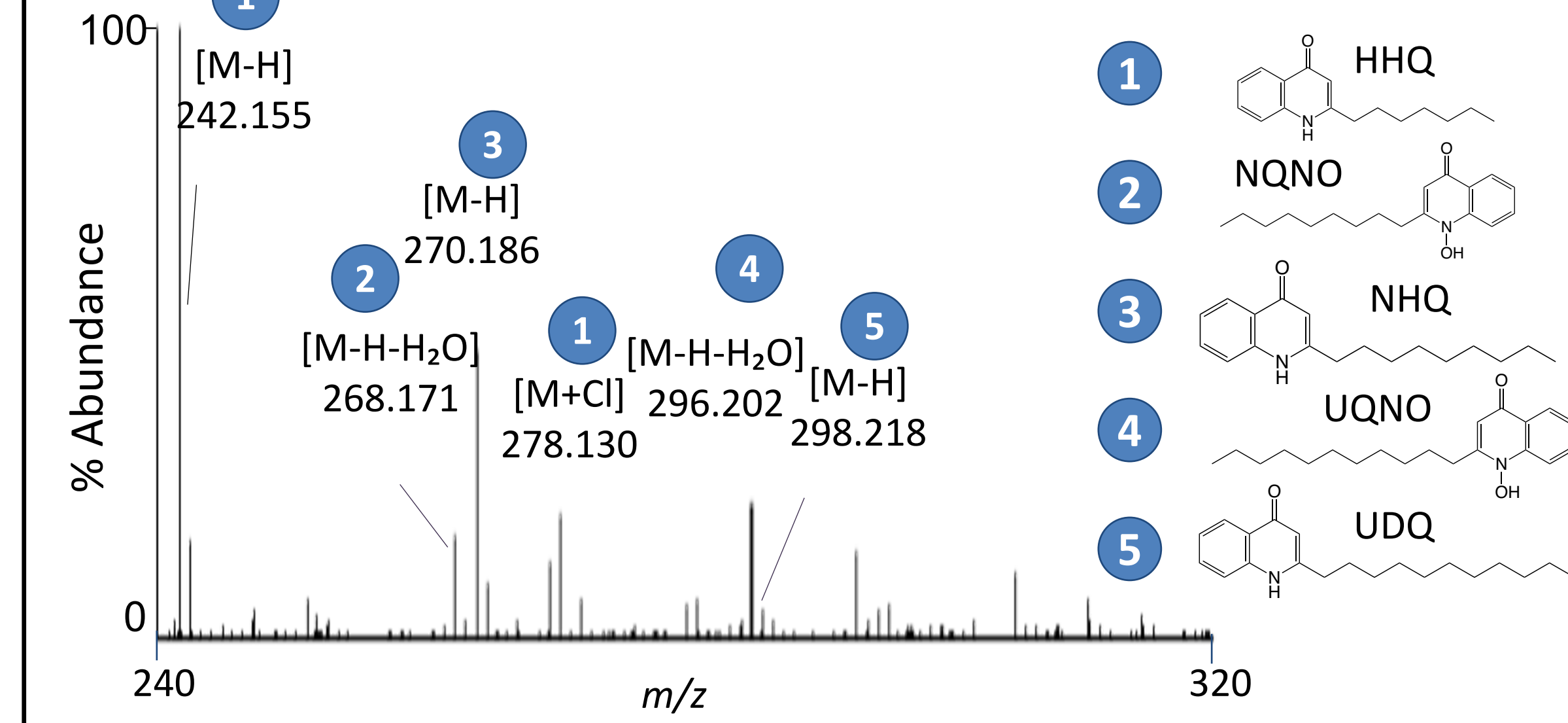
Common bacterial pathogens have distinct molecular profiles



- Rich profiles of small metabolites, fatty acids, lysophospholipids, and glycerophospholipids were observed in all species analyzed.
- PE's were observed at a higher relative abundance in Gram negative organisms while PG's and CL's were more abundant in Gram positive organisms, reflecting plasma membrane composition of these two types of bacteria.⁴
- Many molecular species observed including several glycerophospholipids, quorum sensing molecules, and peptidoglycan precursor UDP - acetylmuramoyl - alanyl - diglutamyl - lysyl - alanyl - alanine are uncommon or absent in human metabolism.

- Amino Acids, Dipeptides, & Other Small Metabolites
- Lysophospholipids and Fatty Acids
- Phosphatidylglycerols (PG)
- Phosphatidylethanolamines (PE)
- Cardiolipins

Quorum sensing molecules observed in *P. aeruginosa*



- Several quorum sensing molecules involved in biofilm formation were observed in *P. aeruginosa* including hydroxyheptylquinoline (HHQ) which is correlated with *P. aeruginosa* pathogenicity.⁵

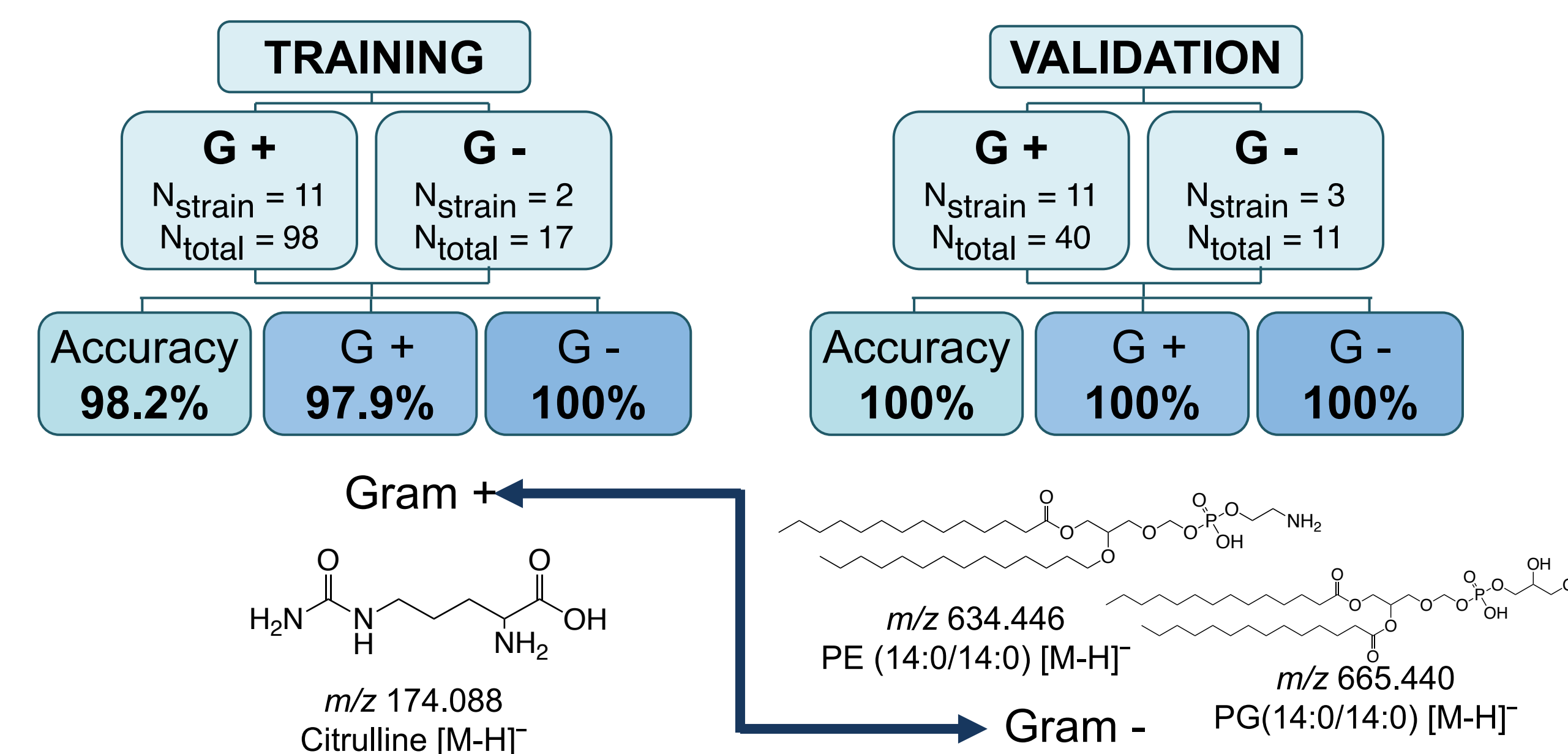
CONCLUDING REMARKS

- Rich molecular profiles that vary between species were observed including trends in glycerophospholipids mirroring membrane composition.
- Quorum sensing molecules that are biomarkers of virulence were observed in *P. aeruginosa*.
- These molecular profiles, when used in tandem with the lasso statistical classification algorithm were predictive of Gram type, genus, and species with 83-100% accuracy for all classification models.
- These results suggest that the MasSpec Pen is a promising technology for clinical identification of bacteria from culture isolates.
- Several molecular species were observed that are absent or uncommon in human metabolism suggesting that the MasSpec Pen has potential to identify bacterial pathogens directly from human biospecimens.

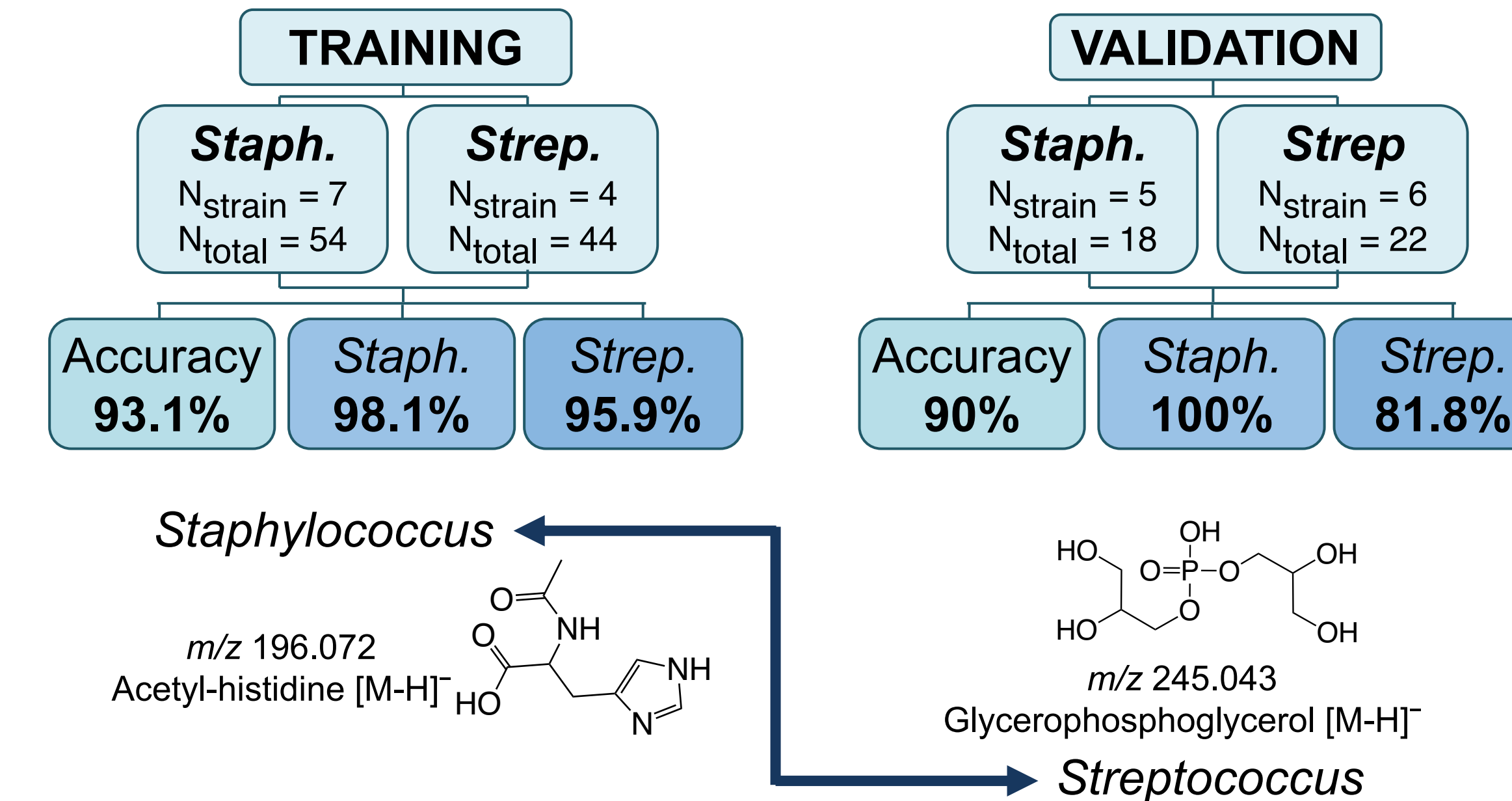
Molecular information is predictive of Gram type, genus, and species

- 83-100% accuracy was achieved for lasso statistical classifiers in both training and validation sets for Gram type, *Staphylococcus v. Streptococcus*, Group A *v.* Group B *Streptococcus* and *S. aureus v. S. epidermidis* indicating that the molecular information obtained by the MasSpec Pen is predictive of Gram type, genus, and species.

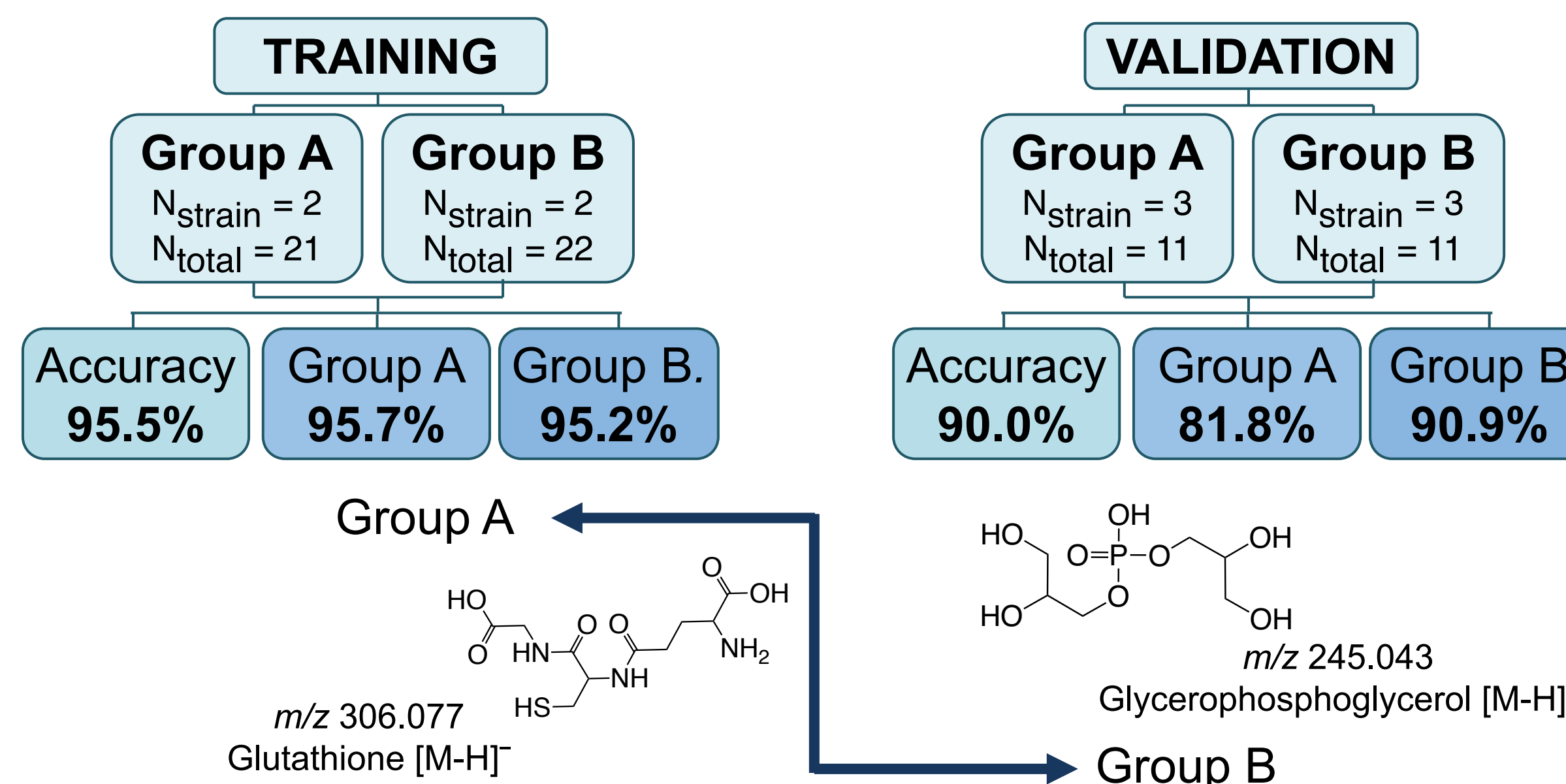
Gram Type



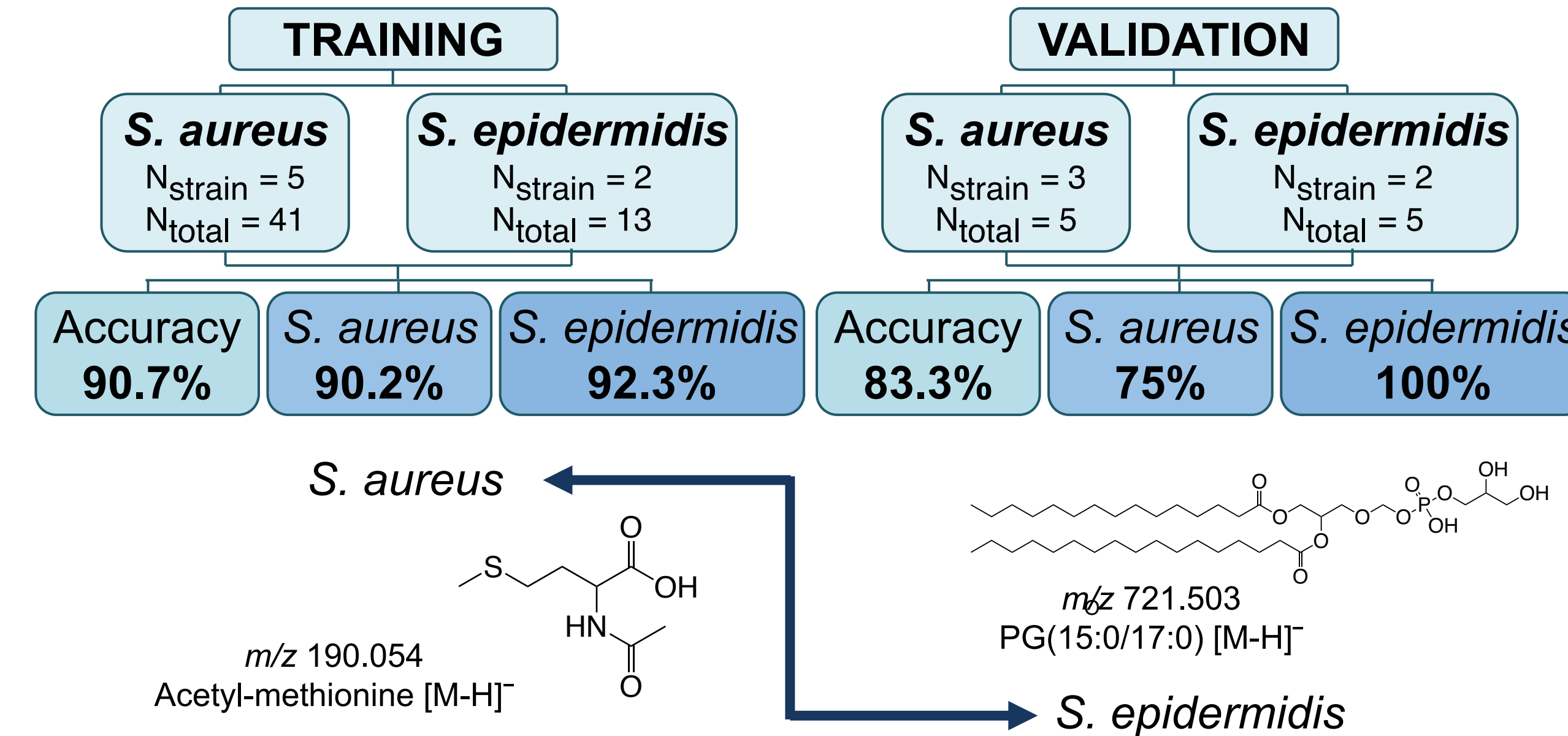
Staph. vs. Strep



Group A vs. B Strep.



S. aureus vs. *S. epidermidis*



ACKNOWLEDGEMENTS

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