**Direct Microbial Analysis by Ambient Ionization MS: Paper Spray and Swab Touch Spray**

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Mass spectrometry has potential to improve patient outcomes and clinical medicine by improving microbial identification. Ambient ionization allows for the rapid analysis (<30 s) of bacteria and fungi with minimal sample preparation. The detection of characteristic biomolecule profiles (*e.g.* lipids) allow for molecular-based identification. Two methods of ambient ionization, paper spray and swab touch spray, are discussed for this application.

Paper spray (PS) ionization allowed differentiation of various species of bacteria and yeast. A small sample (a few micrograms) was taken directly from a culture plate, applied to a triangular paper, and ions were generated upon solvent and high voltage application. Sixteen bacteria species were able to be differentiated. Gram-positive bacteria investigated were easily differentiated, whereas Gram-negative bacteria were more difficult and required the fusion of negative and positive mode data. An analogous PS-MS method was created for the analysis of fungi including eight *Candida* species, representing as much as 95-98% of all the bloodborne yeasts, which were correctly identified ~90% of the time. The simplicity, speed, and *in vitro* performance of PS-MS should allow for extension into clinical applications.

Swab touch spray (STS) is a new ambient ionization method which provides the ability to sample and detect microbes directly from medical swabs. A single bacterial colony was sampled from culture using a swab, affixed in front of the mass spectrometer, solvent and high voltage were applied, and ions were generated via an electrospray-like process. Solvent could be added in discrete additions providing signal pulses or continuously using a syringe pump which provided signal for more than 1 min. The detection of bacteria was demonstrated *in vitro* using clinically-relevant *Streptococcus* species, allowing differentiation of *S. pyogenes*, the primary bacterium that causes strep throat, and related *S. agalactiae*. Lipids corresponding to *S. pyogenes* were detected in simulated throat swabs (comprised of human oral fluid spiked with *S. pyogenes*) representing the initial step toward *in vivo* analysis. PS and STS methods for microorganism

identification are envisioned to extend into clinical medicine, which would have significant impact on patient treatment.