Transformative Proteomics in the Clinical Microbiology Laboratory: MALDI-TOF Mass Spectrometry

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Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) enables inexpensive identification of bacterial and fungal colonies growing in culture in a matter of minutes. This new technology, the product of advances in mass spectrometry as well as bioinformatics and database development, is rapidly changing bacterial and fungal identification in clinical laboratories. MALDI-TOF MS itself was invented in the 1980's and enabled whole organism proteomic analysis. In the 1990's, software for bacterial and fungal identification using MALDI-TOF MS and libraries of reference spectra were developed. Over the past few years, the technology has been commercialized for use in clinical laboratories and is being rapidly adopted globally.

"MALDI" refer to the Matrix, which Assists in the Desorption and Ionization of microbial analytes through pulses of energy from a Laser. The matrix isolates microbial molecules of interest (highly abundant proteins) from each other and enables desorption and ionization of microbial proteins as a result of being "shot" by a laser. The ionized microbial proteins pass through an electrostatic field into a Time Of Flight or "TOF" mass analyzer, a pressurized tube in which ions travel toward a detector. Small analytes travel fastest, followed by progressively larger analytes; as ions collide with the detector, a mass spectrum is generated based on charge and time to impact, representing the number of ions hitting the detector over time. The isolate's mass spectrum is compared to a database of spectra, scoring the relatedness of the spectrum to spectra in the database. A list of most closely-related organisms is generated, each with a numeric ranking showing the level of assurance in identification. Depending on how high the value is, the organism is identified to the genus-, or species-level.

Clinical microbiology MALDI-TOF MS applications test whole bacterial or fungal colonies, without elaborate specimen preparation and using minimal consumables, providing rapid and

cost-effective bacterial and fungal identification. Testing typically begins by "picking" a colony from a culture plate to a "spot" on a MALDI-TOF MS target plate. The cells may be treated on the target plate and are ultimately overlain with of a small amount of matrix. Following a short drying period, the plate is placed in a mass spectrometer. Mass spectrometry and data analysis are automated. Commercial MALDI-TOF MS systems, available from Bruker Daltonics Inc. and bioMérieux Inc., differ in databases, algorithms used to identify organisms and instrumentation.

MALDI-TOF MS improves turnaround time to reporting bacterial and fungal identification by an average of more than a day, compared to standard methods. It reduces reagent and labor costs. Although susceptibility is not directly determined, by rapid organism identification, intrinsic antimicrobial resistance characteristic of particular species (or expected susceptibility based on local antibiograms), may guide therapy. Some investigators have incubated bacteria with antibiotics and used MALDI-TOF MS to measure antibiotic degradation. Since MALDI-TOF MS may determine strain type, strains of S. aureus which are likely to be methicillin-resistant or of Bacteroides fragilis which are cfiA-positive may be detected as a surrogate for resistance. Rapid identification of microorganisms growing blood culture bottles is also a promising application. This requires preparatory specimen processing since blood culture bottles contain non-bacterial sources of protein and other macromolecules which may interfere with microbial identification.