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Oral Session
PCR and other molecular tests directly on blood: what is new?

DEVELOPMENT AND INITIAL CHARACTERIZATION OF A PCR/ESI-MS ASSAY CAPABLE OF DETECTING AND IDENTIFYING DIVERSE BACTERIA AND CANDIDA IN NORMALLY STERILE BODY FLUIDS AND TISSUES.

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Objectives: The diagnosis of sterile-site infections by traditional approaches is often challenging, due to the limitations of culture methods. A PCR/electrospray ionization mass spectrometry (PCR/ESI-MS) assay capable of detecting and identifying diverse bacterial pathogens and *Candida* species in body fluids and tissues is being developed. The system utilizes bead-beating, chemical lysis, and magnetic particle DNA extraction to isolate nucleic acids from specimens. PCR amplification using broad-spectrum primers is then used to amplify DNA fragments from phylogenetically diverse targets. Mass spectrometry analysis of the resulting amplicons followed by database-derived signature matching is used to identify target organisms. In the work described here, the analytical sensitivity of the assay was estimated, the robustness of that sensitivity was tested using biochemically diverse tissues and body fluids, and the assay was compared to traditional culture methods using human specimens.

Methods: The analytical sensitivity of the system was tested using spiked bacteria in muscle tissue, synovial fluid, and human blood. The assay's usefulness in diverse tissues was further confirmed in a variety of tissues and body fluids spanning the range of physiological composition. De-identified (waste) human clinical specimens were then tested and the results of the developed test compared to clinical culture results.

Results: It was determined that the assay performed optimally when using tissue samples of 25-35mg, and non-blood body fluid specimens of 500ul. The lower sensitivity range of the assay, tested in synovial fluid, tissue, and human blood, was determined to be between 50 CFU/sample and 500 CFU/sample for 38 bacterial species and 2 *Candida* species representing commonly occurring pathogens. In testing of 111 clinical tissue and fluid specimens, the assay yielded a 72% positive agreement rate with culture results at the level of individual organism identification, and an 89% positive agreement at the detection (positive/negative) level. The assay yielded negative results in 22/23 tissue specimens (96% specificity) from asymptomatic patients (controls undergoing surgery for noninfectious conditions, presumed to be uninfected).

Conclusion: The method described here is capable of detecting diverse bacteria and *Candida* in a variety of body fluids and tissues, and is able to identify the majority of organisms identified by traditional culture methods in much less time (approximately 6 hours). The utility of molecular techniques such as this should be further investigated through prospective clinical studies.