James B Day, J Nutr Food Sci 2017, 7:3 (Suppl)
http://dx.doi.org/10.4172/2155-9600-C1-041

Bio-Plex suspension array immuno-detection of *Listeria monocytogenes* from lettuce and spinach using virulence protein inducing charcoal-activated enrichment media

James B Day
U.S. Food and Drug Administration, USA

Statement of the Problem: *Listeria monocytogenes*, the causative agent of listeriosis in humans, is a gram-positive bacterium that is contracted via the consumption of contaminated foods. Various leafy green vegetables, including lettuce and spinach, have been implicated in human listeriosis cases. Molecular methods and immuno-based techniques for detection of *L. monocytogenes* in these food matrices can be difficult due to the presence of assay inhibiting elements.

Methodology: In this study, we utilize a novel enrichment media containing activated charcoal as the key ingredient that induces overexpression and secretion of *L. monocytogenes* virulence proteins. The Bio-Plex suspension array system, based on Luminex xMAP technology, can then be utilized to specifically detect accumulated *L. monocytogenes* virulence proteins via a magnetic bead-antibody complex. Iceburg lettuce and packaged ready-to-eat spinach were treated with *L. monocytogenes* and incubated in preenrichment broth (Buffered Listeria Enrichment Broth) followed by incubation in charcoal activated media. The supernatant fraction was TCA precipitated and *L. monocytogenes* lysteriolysin O (LLO) was collected using magnetic microspheres conjugated to LLO specific antibody. A newly developed antibody that exclusively recognizes *L. monocytogenes* LLO was used as the biotin conjugated secondary antibody and analysis was conducted using the Bio-Plex 200 system.

Findings: As few as 1 CFU/ g of *L. monocytogenes* were detected in both foods tested. Whole cell fractions from 14h activated charcoal enrichments were also analyzed using antibody that recognize both pathogenic and non-pathogenic *Listeria species* which also resulted in a detection limit of 1 CFU/ g. Internal control beads were also utilized to ensure proper instrumentation function, integrity of assay reagents and to eliminate the possibility of non-specific interactions.

Conclusions & Significance: This method is the first to specifically recognize and differentiate *L. monocytogenes* among other nonpathogenic *Listeria species* in various leafy greens using immune-detection. The total presumptive detection time can be achieved in less than 24h.

Biography

James B Day is a Research Microbiologist at the U.S. Food and Drug Administration in College Park, Maryland, where he is involved in developing detection methodologies for bacterial pathogens in contaminated foods. He has developed techniques for rapid identification of *Francisella tularensis, Salmonella enterica and Listeria monocytogenes* in various food matrices and recently established a novel macrophage-based assay for enrichment of intracellular bacterial pathogens for enhanced identification. He earned his PhD from the University of Miami School of Medicine (UM), where he worked on bacterial pathogenesis of *Yersinia pestis*. At UM, he developed a widely used system to measure virulence protein secretion and host cell translocation. He went on to complete his Postdoctoral studies at Harvard Medical School, where he worked on type III secretion mechanisms of *Salmonella enterica* as well as regulatory factors that control virulence protein induction.

Email: james.day@fda.hhs.gov

Notes: