Background
The following is a list of high priority *Listeria* research topics that are of interest to the produce industry, as data generated from these identified research topics would greatly aid produce business operators in their efforts to control, reduce or eliminate the risk of listeriosis associated with produce consumption. This DRAFT was compiled by the PMA / United Fresh Joint Listeria Working Group that convened on 13 September 2016 in Washington, DC. The PMA / United Fresh Joint *Listeria* Working Group is composed of a wide range of produce safety professionals from all segments of the produce supply chain. This research list is intended to inform produce safety researchers and competitive research grant programs such as USDA NIFA and the Center for Produce Safety (CPS) about current produce industry research needs. The following research topics are not ranked in order of research priority or importance. In addition to research topics, the PMA / United Fresh Joint *Listeria* Working Group also discussed means of enhancing communications between the produce industry and produce safety researchers. Communication recommendations for consideration are also outlined below.

Research Topics

I. Prevalence & Persistence of *Listeria monocytogenes* (Lm)
   - To inform risk based decision making, research is warranted to determine the prevalence and persistence of Lm in various environments and micro-environments throughout the produce supply chain including but not limited to:
     - Produce on farm / orchard at the time of harvest,
     - Packinghouse and warehouse dry environments,
     - Produce coolers and distributions centers and
     - Produce Micro-environments (e.g. HVAC units and motors which created elevated temperature micro-environments; condensation that creates a wet surface in an otherwise dry operation, etc.)
   - Determine procedures, practices and factors that influence the rate of “resident” Lm establishment on produce farms, and in harvest operations, packing operations and fresh-cut produce processing plants.

II. Basic Biology
   - Lm virulence: It has been demonstrated that Lm serotypes can and do vary in their virulence and human pathogenicity. Understanding Lm serotype virulence and pathogenicity is critical to understanding the public health burden and risk
attributable to various Lm serotypes that may be isolated on produce or in produce handling environs. This research may open the possibility of establishing an acceptable level of specific Lm serotypes in some foods, IF it is determined that some specific Lm serotypes are non-pathogenic or require extraordinarily high doses, due to growth in foods, to cause human morbidity or mortality.

- **Understanding Listeria spp as Index Organisms for Lm Presence**: There are currently 17 known Listeria species. It is important to understand which L spp persist by establishing niches and harborages in produce growing, packing and fresh-cut processing environments and correlate with the presence or absence of Lm. This information may assist developers of rapid detection technologies to develop more precise environmental monitoring tools based on the use of index organisms specifically for the produce industry.

- **Lm Growth or No Growth in Foods**: Determining which raw agricultural commodities and fresh-cut produce products will support the growth of Lm at recommended and abusive storage temperatures as this is an important consideration regarding public health risk from a contaminated food product. This information is important to assess the potential for Lm proliferation which is an important factor in assessing public health risk and helps inform the entire produce supply chain regarding safe handling best practices.

- **Differentiation of “Transient” vs “Resident” Lm**: Development of rapid assessment tools to enable produce handlers to differentiate between “transient” and “resident” Lm in their facility and on food contact surfaces is needed. These type of tools would greatly assist produce handlers in assessing and verifying their sanitation preventive controls.

- **Metagenomics**: Produce, produce farms, harvesting, packing and processing environments have diverse microbial communities. Understanding how these diverse and often un-culturable communities of microbes may enhance or reduce the potential for establishment of “resident” Lm by for example competitive exclusion, may lead to better environmental monitoring strategies or use of microbial antagonists to prevent or reduce the likelihood of the establishment of “resident” Lm.

### III. Detection

- **Lm Culture vs Molecular Techniques**: Human pathogen culture methods have long been regarded as being the “gold standard” for human pathogen testing. However, new molecular techniques are as accurate and in some cases even more sensitive in their ability to detect human pathogens such as Lm. Comparison of various new sampling and enumeration techniques are warranted with particular emphasis on produce food matrices, inputs used for produce production and commonly used produce harvesting, packing and processing food contact surface materials.

- **Lm Genetic Drift**: Understanding factors that affect the rate of Lm genetic drift (rate of genetic change over time as determined by Whole Genome Sequencing) as influenced by various produce growing, harvesting, packing and processing environments is important to understand, assess and evaluate if Lm isolates from
environmental samples are “transient” or “resident”. Additionally, this information will provide insights when assessing the relatedness of for example clinical isolates form ill person(s) and environmental isolate(s) from produce environments.

IV. Lm Risk Assessment

- **Risk-based Corrective Actions:** Currently, commonly used *Listeria spp* and Lm environmental monitoring program test techniques are qualitative or semi-quantitative. Development of quantitative *Listeria species* and/or Lm environmental monitoring technologies would be helpful to assist operators to prioritize and determine the magnitude of corrective actions when Lm or *Listeria spp* positive test results occur.

- **Equipment Risk Assessment and Sanitation Frequency:** If equipment is of less-than-optimal sanitary design and cannot easily be refurbished or retrofitted, effective cleaning methods and frequencies should be determined to minimize risk. This would include an evaluation and prioritization of different pieces of equipment based on persistence data and the rate at which Lm can establish residence in/on different pieces of equipment. Cleaning frequencies and methods could be established that reduce the likelihood of Lm establishing residence.

- **Rate of Transference in Produce Facilities:** Conventional thinking is that finding a *Listeria* positive in Zone 4 is of lower risk than finding in Zone 3; that Zone 3 is lower risk than Zone 2, etc. Understanding how *Listeria* travels through a facility can aid in the development of optimal facility design and environmental testing strategies.

V. Lm Preventive Controls (Cleaning & Sanitation / Interventions)

- **Phages:** The use of *Listeria spp* or Lm specific phages to control the establishment or growth of Lm in produce growing, packing and fresh-cut operations is warranted.

- **Dry Cleaning:** As some produce unit operations and machinery are not currently designed for wash down cleaning and sanitation (including some electronic devices), research and development of effective means to dry-clean facilities including produce food contact surfaces in packing house operations and fresh-cut produce processing environs is warranted.

- **Sanitary Design Retrofitting:** Currently some produce establishment food contact surfaces may be of less-than-optimal sanitary design and thus allow for Lm to establish niches and harborages. This equipment is a significant capital expenditure for packinghouse and fresh-cut processors. Therefore, development of options to inexpensively and effectively retrofit or refurbish such equipment to eliminate such niches is warranted. An example of such technologies may be use of rechargeable anti-microbial coatings that prevent the establishment or growth of Lm on equipment surfaces.

- **Sanitizers:** Investigation is warranted to identify chemical sanitizers (conventional, green and organic) that are effective at disinfecting common food contact surfaces found in raw agricultural commodity packing houses such as
wood and painted metal surfaces. Additionally, as hot water may not be universally available at all produce operations for cleaning and sanitation, the effect of temperature on the efficacy of various aqueous based food contact cleaning and sanitizing chemicals is warranted.

Communications

- **Clearing House of Information:** It was suggested that a clearing house of information and knowledge regarding Lm and its control be established to assist in knowledge sharing across industry sectors.
- **Case Studies:** It was suggested that case studies be developed which provide detailed information including root cause analysis of contamination events which led to foodborne illness outbreaks and/or product recalls due to Lm contamination of fresh produce. These case studies would be informative for the produce industry businesses to understand what contributing factors may have led to these contamination events and provide them with an opportunity to take appropriate preventive measures to prevent such incidents in the future.
- **Guidance to the Research Community:** It was suggested that a research forum (e.g. in-person meeting, webinar, etc.) between produce business food safety professionals and the produce safety research community be established to inform produce safety researchers about:
  - Current ongoing industry practices and any industry wide initiatives that are in progress to enhance produce food safety and reduce the potential risk of listeriosis from produce.
  - The importance of using real world conditions, for example use of scuffed / worn food contact surfaces for research to assure that research outcomes are highly applicable to the produce industry business operators,
  - Differences between various culture versus molecular methods for Lm detection and enumeration which may bias research with spurious results.
- **Compilation of Ongoing Lm Research:** It was suggested that a list of publically funded ongoing Lm research be compiled and be posted to reduce the potential for redundant Lm research efforts.