Thank you for participating in the webinar “Listeriosis in South Africa – Lesson Learned.”
We received lots of questions during the webinar and we appreciate your engagement and apologize for not being able to answer all of questions during the session due to time constraints. Speakers and organizers of the webinar have reviewed questions and put together a Q&A sheet. We compiled some of similar questions that were raised several times.

Questions related to *Listeria* controls and testing.

**Q:** Are there any recommended control procedures/ corrective actions for *Listeria monocytogenes* at RTE food facility?

**A:** Good Manufacturing Practices (GMPs), or Good Hygiene Practices (GHPs), are the base of any food safety programs and systems. In the United States, the Food and Drug Administration (FDA) regulated foods are under the Current Good Manufacturing Practice, Hazard Analysis and Risk-Based Preventive Controls for Food for Humans regulation, 21 CFR § 117 (PCHF). Subpart B of this regulation deals with current GMPs (cGMPs). The U.S. Department of Agriculture’s Food Safety Inspection Service (USDA FSIS) developed Sanitation Standard Operating Procedures (SSOPs), 9 CFR § 416. For further food safety programs beyond GMPs/GHPs, a majority of US food manufacturing facilities are required to have Hazard Analysis Critical Control Point (HACCP) and/or PCHF Food Safety Plans. Both programs address control of food safety hazards (such as *Listeria monocytogenes*) based on the outcome of a hazard analysis. In the later program, there is more emphasis on the control of environmental pathogens (including *L. monocytogenes*) for ready-to-eat (RTE) foods. FDA recently developed the “Draft Guidance for Industry: Control of *Listeria monocytogenes* in Ready-To-Eat Foods,” the requirements of which closely parallel FSIS’ “Compliance Guidelines: Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-To-Eat (RTE) Meat and Poultry Products.” The FSIS guidelines brought success to the meat and poultry industry in controlling *Listeria* contamination and a similar approach by FDA should aid the entire industry in controlling *Listeria*. Similar regulations have been developed in other countries. There are many more excellent references for controlling *Listeria* in food facilities. Some of them are listed below. These guidance and publications discuss from fundamental food safety programs, control strategy of *L. monocytogenes* and corrective actions.

- **FDA PCFH and Draft Guidance:** [https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm](https://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334115.htm)
- **FSIS SSOP:** [https://www.fsis.usda.gov/wps/wcm/connect/4cafe6fe-e1a3-4fcf-95ab-bd4846d0a968/13a.IM_SSOP.pdf?MOD=AJPERES](https://www.fsis.usda.gov/wps/wcm/connect/4cafe6fe-e1a3-4fcf-95ab-bd4846d0a968/13a.IM_SSOP.pdf?MOD=AJPERES)
- **FDA HACCP Regulations and Guidance:** [https://www.fda.gov/Food/GuidanceRegulation/HACCP/FDA](https://www.fda.gov/Food/GuidanceRegulation/HACCP/FDA)
- **Draft Guidance for Industry:** Control of Listeria monocytogenes in Ready-To-Eat Foods: [https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm073110.htm](https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm073110.htm)
Are there any recommended methods for environmental monitoring programs (development, sampling methods, and tools etc.)?

AI: In general, the environmental monitoring program is to collect and monitor baseline microbiological data at food manufacturing facility. Importantly the program is to verify whether cleaning and sanitation programs (and other GMPs/GHPs programs) are working. It has been recognized that if any abnormal results (e.g., high counts in a specific location compared to past data at the same location or area) are found, the operator may assume a hygienic program (e.g., cleaning/sanitizing practices) was not successful and take appropriate corrective actions (i.e., additional cleaning, or changing employee’s traffic patterns etc.). Inclusion of environmental programs and/or pathogen environmental programs to your food safety systems/programs can be decided through a risk based analysis (e.g., hazard analysis). It may be necessary to include environmental pathogen monitoring as a verification measure when a hazard analysis concludes that contamination by environmental pathogens are a reasonably foreseeable hazard (e.g., Listeria environmental monitoring program at RTE areas for RTE products that are exposed to environment after cooking process). For development of environmental program, guidance documents (e.g., FSIS guidelines and FDA’s draft guidance mentioned-above) can be referred - Listeria control and environmental monitoring programs are often discussed in the same document since they are interconnected. Also, other excellent publications are listed below.


Should we include Listeria environmental monitoring program for dry processing facility?

As mentioned above, decisions to include pathogenic environmental monitoring program is based on outcomes of risk analysis (generally a hazard analysis). Risk of contamination and growth capability of L.
*monocytogenes* depends on the food facility, food safety programs, ingredients, processing methods, formulation of products, and intended customers, to name a few. Typically, dry food facilities are focused more on *Salmonella* environmental programs. Wet areas within a dry facility may require close analysis to decide whether to include *Listeria* in their environmental monitoring programs. The references below may help when you conduct hazard analysis and understanding *Salmonella* control in low moisture food facility.


**Question related to infectious dose and threshold of *L. monocytogenes*.**

**Q: What is the infectious dose of *L. monocytogenes*?**

**Scott:** The infectious dose of *L. monocytogenes* is not fully understood. Dose-response relationships for the pathogen were summarized by FAO/WHO in their Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods. Factors that contribute to the uncertainty include incomplete epidemiological information, uncertain extrapolations from animal data to humans, the absence of human feeding trial data, lack of mechanical models, and insufficient understanding of strain variation and food matrix effects.


**Q: What are the microbiological criteria for *L. monocytogenes* in ready-to-eat foods?**

**Scott:** Codex Alimentarius established microbiological criteria for *L. monocytogenes* in ready-to-eat foods whereby 100 CFU/g is the limit (n=5, c=0) in ready-to-eat foods that do not support the growth of the pathogen. In ready-to-eat foods in which growth of *L. monocytogenes* will occur, the absence of the pathogen in 25-g samples (n=5, c=0) is established.

**Codex Committee on Food Hygiene.** 2009. [http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCAC%252BGL%252B61-2007%252FCXG%252B061e.pdf](http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCAC%252BGL%252B61-2007%252FCXG%252B061e.pdf)

**Questions related to the *Listeria* strain**

**Q: Were the 43 CDC SA isolates from the current outbreak? If so, are they clinical or food isolates?**

**Henk:** The 43 CDC SA isolates were from the SA outbreak (I confirmed this with my colleagues at the CDC). Metadata for these isolates is not publicly accessible.

**Q: Do you have any information regarding the products identified to contribute to Listeriosis cases, that are not ST6**

**Henk:** I have no data on the source of these non-ST6 isolates.

**Q: It seems that the Western European ST6 and South African strains are clonally related based on the WGS data. Could it be a matter of transfer by trade/humans, or rather independent arise in the different geographic areas?**

**Henk:** I hope I can cover these questions with one long answer. The WGS analysis presented in the webinar was based on all data for all isolates for CC6 (which includes ST6, ST1292, ST615, ST616, ST823, ST1207) currently in the SRA database at NCBI ([https://www.ncbi.nlm.nih.gov/sra/](https://www.ncbi.nlm.nih.gov/sra/)), currently 839. This dataset is highly skewed toward isolates from the United States (561 accessions), and Western Europe (> 100 accessions). This analysis shows that while the ST6 accessions from South Africa are distinct by 14 SNPs from all other ST6 accessions, they are firmly nested in a subpopulation of ST6 accessions that are mostly from Western Europe (The closest accessions are from United Kingdom and the Netherlands). I would not say the SA isolates are ‘novel’, since they are extremely similar to the Western European
isolates. From what I can tell there is no further epidemiological evidence linking these accessions to the SA outbreak. Given this close relationship, and the number of SNPs it is easy to speculate that the ancestors of the SA outbreak strain have been introduced from Western Europe sometime in the past, potentially with ingredients, equipment, etc. The caveat, however, is that we need a lot more (WGS) data on the distribution of CC6 in South Africa and other parts of the world to confirm this scenario with any certainty.

Q: the HIV status and pregnant women in the outbreak seems to contra indicate the result from the French work.
Henk: I think we are getting caught in (statistical) semantics. The French paper said CC6 in France was ‘was more prevalent among patients with ’...few or no immunosuppressive comorbidities...’, as compared to other CCs. It does not say that immunocompromised individuals were not affected by CC6 in France, just that relatively more non-immunocompromised people were affected by this CC. I do not think that constitutes necessarily a contradiction.

Q: In the session, dr. Den Bakker discussed about a rare Listeria strain with a quaternary ammonium compound resistance gene. How much of this strain was responsible for the illnesses and deaths associated with the outbreak
Henk: I meant a rare mobile element (which turns out to be a plasmid) which confers potential quat resistance for Listeria monocytogenes host. I found this plasmid in 12 of the SA outbreak strains, and in 56 Western European isolates. The fact that this mobile element is not found in all isolates may either be an artifact of isolation, DNA extraction and sequencing (plasmids are easily lost during L. monocytogenes enrichment etc.), or may be biological (an unstable plasmid, secondary factors that may to loss of the plasmid).

Q: Is there any sequencing database for strains from foodborne illness incidents/outbreaks.
Ai: In the United States, FDA developed public database called GenomeTraker (link below), together with the National Center for Biotechnology Information (NCBI) to upload sequencing data including strains from foodborne illness incidents. FDA, FSIS, Centers for Disease Control and Prevention (CDC) have been uploading data onto the database.
FDA GenomeTrakr Network:
https://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/ucm363134.htm