news digest #016

Chasing *Listeria* in Food and Water: Advancements in detection and strain discrimination

As winter approaches in Europe, North America and Asia, ushering in the season of holidays like Christmas and New Year, we also welcome some delightful seasonal treats, such as the timeless classic, the red candy apple. But are candy apples entirely safe to consume, or are we potentially overlooking health risks, unwittingly venturing into a culinary danger zone? Let's delve deeper into this matter.

The candy apple, wrapped in its sugary caramel coating, conceals a potential risk beneath its sweet facade. Underneath this sugary layer, moisture lurks, creating an ideal breeding ground for microorganisms like *Listeria*. The insertion of a wooden stick into the apple can draw out a small amount of juice from the fruit. It's crucial to remember that *Listeria* strains can grow on the skins of various fruits, and when the apples are pierced with the wooden skewers, this can push *Listeria* from the apple's exterior into its core.[1]

While candy apples are relatively less frequently consumed and often associated with local markets, what about globally prevalent and abundant products like ice cream, leafy greens, or brie and camembert cheese? Surprisingly, all these foods have been linked to multistate outbreaks during 2022-2023 in the United States.[2]

For instance, the Food and Drug Administration (FDA), in collaboration with the Centers for Disease Control and Prevention (CDC), supported the Florida Department of Health (FL DOH) and Florida Department of Agriculture & Consumer Services (FDACS) in investigating an outbreak of *Listeria monocytogenes* infections connected to ice cream from a major manufacturer. This outbreak resulted in 28 illnesses across 11 states, and on November 2, 2022, it was declared to be over. Experts collected environmental samples from the production facility and samples of finished products, successfully identifying the outbreak strain in both the ice cream and its production environment.

Another severe outbreak of listeriosis, caused by *Listeria* bacteria, was identified in South Africa in 2017, where out of 728 patients with a known outcome 193 (27%) died. When health authorities initiated the collection of food samples from the homes of patients in November 2017, *L. monocytogenes* was isolated from a food sample, and a trace-back investigation was conducted.[3] Investigations showed that in a middle-income country with a high prevalence of HIV infections, *L. monocytogenes* caused disproportionate illness among pregnant girls and women and HIV-infected persons. Today, many countries are placing a strong emphasis on early and rapid detection of *Listeria* and source tracking. This approach enhances the monitoring of frequently recalled products like seafood and meat, and significant progress has been made in the past three decades.[4]

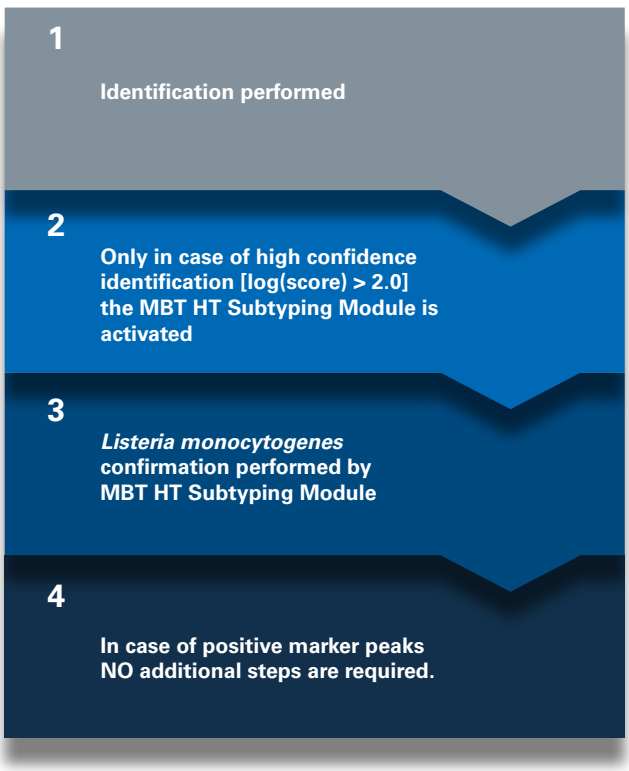
In 2021, listeriosis ranked as the fifth most reported zoonosis in the European Union, following *Salmonella* and *E. coli*. Surprisingly, *Listeria*, despite being a significant foodborne pathogen, often remains overlooked in the public eye.[5]

Beyond food, the contamination of water used in post-harvest handling and processing of fresh and frozen produce, such as fruits, vegetables, and herbs, is a global concern. *L. monocytogenes*, alongside *Salmonella*, Enterococci and Coliforms, is among the most relevant microbial hazards associated with water used in various processing stages.[6]

Listeria primarily affects individuals over the age of 60, pregnant women, newborns, and those with weakened immune systems.[7] What makes *Listeria* unique for microbiologists is its ability to thrive in salty environments and at low temperatures (between +2°C / 35°F and +4°C / 39°F), unlike many other foodborne bacteria. This resilience, coupled with the high mortality rates associated with *Listeria* infections, underscores the critical importance of safe food and water handling. The *Listeria* genus comprises more than 15 species, but *L. monocytogenes* is the primary culprit behind human infections, while *L. ivanovii* is pathogenic in animals but rarely affects humans.[8] *L. monocytogenes* can form biofilms, providing a haven for microorganisms and aiding their resistance to external stresses.

European Union Regulation 2073/2005 establishes food safety criteria for important foodborne bacteria, including *L. monocytogenes*. Various methods, such as traditional plating according to ISO 11290 or specific PCR kits incl. a pre-step, DNA extraction from liquid pre-enrichment with various food matrices and subsequent PCR amplification for *Listeria* spp. and *L. monocytogenes* specific elements, can be used for detection.[9] Linear MALDI-TOF MS has emerged as a rapid method for identification and confirmation of *Listeria* spp. or *L. monocytogenes* specifically, starting from colony material as shown in dedicated studies in 2018.[10] The MALDI Biotyper® uses a hierarchical approach to confirm *L. monocytogenes*.

When using the MALDI Biotyper for the identification of an unknown sample, the mass spectrum of the latter will be measured and compared to the reference library entries. These entries are the so-called Main Spectra (MSP), which represent unique proteomic "fingerprints" of the respective strain. The MSPs for different *Listeria* strains incl. *L. monocytogenes* have been generated by the software of the MALDI Biotyper system, which uses a proprietary algorithm to analyze the mass spectra of different strains and create a unique fingerprint for each of them. The MSPs are stored in the Bruker reference library and used for identification of the samples, including *Listeria* spp. However, in food safety labs a second level of mass spectrum analysis is mandatory and therefore triggered automatically: upon identification of *Listeria* spp. the MALDI Biotyper activates the MBT (HT) Subtyping Module, a crucial software add-on for the differentiation of the closely related *Listeria* species. Confirmation of *L. monocytogenes* according to the validation studies is only possible when the customer follows Bruker's guidelines of the well-established hierarchical approach: in addition to the results report with a high confidence result (shown by green traffic light), there must be confirmation of *L. monocytogenes* with the MBT (HT) Subtyping Module, which automatically detects species-specific marker peaks. If these marker peaks are not identified, the software will guide the user and e.g., (a) recommends repeating the samples with the extraction procedure instead of directly smearing onto the target plate or (b) to repeat with another fresh colony.



Seamless and fast workflow for *Listeria* subtyping.

Identification of specific strains (identified by "serogroups" and "serovars") is important for human health as they can differ in terms of geographical distribution and their ability to cause disease. Detection and isolation of *Listeria* strains, both by classic laboratory procedures and rapid microbiology methods, are therefore crucial to evaluate their relative presence in food or the environment, as well as to address the virulence question and to precisely trace outbreaks by identifying the source of contamination.

Rapid identification of this bacterium at a subspecies level is important for tracing back multiple occurrences and improve risk-based inspection programs. A method for subtyping *L. monocytogenes* at the serotype and haplotype levels is FT-IR (Fourier Transform – Infrared Spectroscopy) technology. Sample analysis with the IR Biotyper® offers a rapid strain discrimination method starting from colony material. This pre-screening tool allows to speed up strain discrimination analysis, providing results that can help to avoid costly Whole Genome Sequencing (WGS) sample analysis. Targeted identification of serogroups with the IR Biotyper has been demonstrated recently by a governmental lab in Southern Germany.[11] In one local validation study, the lab has tested serogroup 1/2 (SG1/2) differentiation to be applied only onto *L. monocytogenes* samples, plus SG3/7 and SG4 in a second and third approach. Out of more than 250 *L. monocytogenes* SG1/2 spectra 100% were classified correctly (inclusivity) to serogroup 1/2 with a new Bruker classifier. This classifier has been created by artificial neural network (ANN) machine learning as a solution for *L. monocytogenes* identification on serogroup level. Additionally, out of more than 350 non SG1/2 spectra 100% were classified correctly (exclusivity) as not SG1/2. The new workflow allows laboratories to validate the discrimination of *L. monocytogenes* serogroups 1/2, vs. SG3/7 or vs. SG4.[12]

Cost-effective solutions like the IR Biotyper can be deployed by third-party or in-house laboratories to rapidly investigate contamination events in the food processing environment. FT-IR with dedicated classifiers such as the described "Listeria Serogrouper" is a fast subtyping method, starting from colony material. It can continue to play a valuable role as a simple, and cost-effective method to identify and track *L. monocytogenes* subtypes e.g., in factory environments and animal breeding facilities. While FT-IR may not possess the same discriminatory power as WGS in identifying persistent clones it proves to be a valuable screening tool, especially in situations or locations where WGS may not be easily accessible to food business operators or to save costs and time as many samples (de-replication approach) do not need to be transferred to WGS .

Outlook: In addition, the MALDI Biotyper can be used for the detection of specific antimicrobial resistances (AMR), a rising topic for *Listeria* strains found in the industry and environmental products. Different recent profiling studies have shown that a significant number of *L. monocytogenes* isolates shows resistance against prescribed/recommended antimicrobials for the treatment of listeriosis and further non-prescribed antimicrobials. Strains have been observed with antimicrobial resistance against classical sulfamethoxazole, amoxicillin, penicillin but also oxytetracycline, cefotetan, ceftriaxone, or streptomycin.[13] This reflects the picture we are seeing in clinical isolates and shows clearly that AMR is an existential threat to humans found in many sectors of our lives.

Summarizing the Bruker *Listeria* workflow, the MALDI Biotyper offers a solution for identifying microorganisms through proteomic fingerprints, particularly beneficial for confirming *Listeria* spp. and *L. monocytogenes*. On the other hand, the IR Biotyper serves as a swift microbiological method for strain discrimination. It includes ready-to-use classifiers, which prove valuable for typing *L. monocytogenes*, based on colony material. However, it's important to note that the IR Biotyper isn't confined to this classifier alone. It supports various other species, allowing users to test their own strain collections. This flexibility extends to constructing dendrograms and scatter plots for strain discrimination across Gram-positive and Gram-negative bacteria, including *Listeria* spp., *E. coli* or *Salmonella* spp. (see graphic on last page).

References

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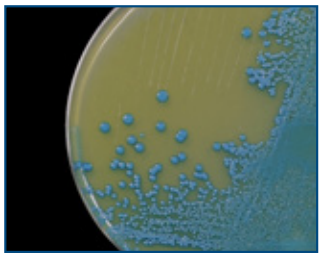
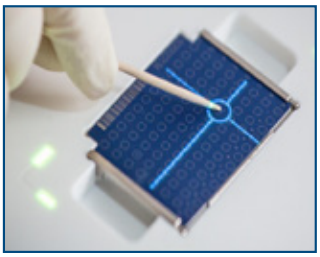
Selection of typical *Listeria* spp. colonies



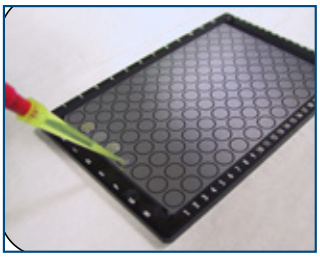
Preparation of sample and control onto MBT Biotarget 96 plate



***Listeria* spp. and *L. monocytogenes* confirmation on the MALDI Biotyper®**

***Listeria* spp. and *L. monocytogenes* confirmation in minutes**

Prepare a homogeneous suspension with the IR Biotyper® Kit



Pipet samples and control onto the target plate, dry the samples

***Listeria* strain discrimination (SG 1/2, 3/7 and 4) on the IR Biotyper®**

Fast strain discrimination (SG 1/2, 3/7 and 4) of *L. monocytogenes*

***Listeria* spp. and *L. monocytogenes* confirmation and *L. monocytogenes* strain differentiation**

Typical workflow for confirmation of *Listeria* spp. and *L. monocytogenes*, followed by strain differentiation using classifiers provided by the IR Biotyper: Starting from colony material and using both instruments in parallel or sequential, *Listeria* spp. and *L. monocytogenes* confirmation and *L. monocytogenes* strain discrimination (SG 1/2, 3/7 and 4) can be achieved very fast, starting from colony material.

Not for use in clinical diagnostic procedures.
Please contact your local representative for availability in your country.

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