Listeria Overview

Listeria, a potentially life-threatening bacterium, is found throughout the environment - in soil, water and possibly on the product/raw material as it enters the facility for processing. It is also found in the intestinal tracts of animals such as cattle. Once introduced into a food processing facility, Listeria can survive in cold, damp environments. While other pathogenic bacteria (E. coli and Salmonella) can grow in cold storage, Listeria is able to survive and flourish, even in the presence of high salt. Common areas where Listeria is found include on walls and plastic strip curtains and in cracks and crevices of equipment. Drains and drainage systems such as cooling units and their drip pans are danger areas. They provide an ideal growing environment for Listeria. There are multiple species of Listeria, but Listeria monocytogenes (L. monocytogenes) is of primary concern as it causes listeriosis which has a high mortality rate in compromised individuals as well as elderly and young.

Currently, regulations mandate environmental surveillance for the presence of Listeria sp. in food processing facilities to minimize the risk of foodborne illnesses associated with contaminated food. Despite this, foodborne illnesses due to Listeria continue to be a challenge in the food production system. Some countries require the absence of L. monocytogenes in raw foods used to prepare RTE food. Nevertheless, up to 30% of raw foods contain Listeria. Therefore, it is critical to have a comprehensive environmental monitoring plan for Listeria that includes containment and risk mitigation criteria. The best approach is to implement surveillance of a large number of locations for Listeria sp. and specifically, L. monocytogenes using a simple, rapid, cost-effective screening method.

Testing for Listeria

The most common tools for Listeria testing are swabs and sponges. Swabs are typically used for food contact surfaces of tighter, hard to reach positions. Sponges are typically used for non-food contact surfaces such as walls and drains. Either can be used throughout the facility. Samples must then be incubated for 24-48 hours to determine if Listeria is present.

However, positive results from these rapid tests are considered “presumptive positives” so further testing is needed to confirm the results. The test results could prove to be false-positive results which means extra costs for food producers in addition to the extra time used to confirm the results. According to ISO/FDA/USDA standards, the process of confirming test results begins...
with streaking selective agar plates from the presumptive positive sample. These agar plates must be incubated for 24 – 48 hours to confirm a true positive result and must be incubated at a different temperature from that of routine samples. Collectively, the overall sample testing process can take 4 – 5 days, and even longer, if sending samples to a third-party lab for testing. This poses some logistical issues: if a lab does not have an additional incubator, the main incubator must be set aside and adjusted to the required temperature (meaning more downtime as the incubator cannot be used for enrichments); holding/quarantining product takes up significant space in the facility while waiting for the final results (taking up valuable space that could be used for other product storage); and selective agar plates have limited shelf-lives (they may not be available immediately for testing, delaying time to results). Overall, a false-positive result costs unnecessary time, labor, and money.

**Overcoming the Time to Results (TTR) Challenge**

Rapid testing technology for foodborne pathogens allows test results to be known by processors and much more quickly, allowing them to release products to the market more rapidly. As noted above, it can eliminate holding products for long periods of time, which can affect both the freshness of products and the cost of operations. While most rapid tests only provide presumptive results, when combined with the latest confirmatory testing, results can be obtained in 2-3 days, saving valuable time and money. An excellent example is detailed below.

**Faster, Accurate Testing Method for Listeria/L. monocytogenes**

Rather than standard culture methods for *Listeria* detection, which can take up to a week, a new approach was developed by combining two rapid tests, the first for the detection of presumptive positives and the second for confirmation of results by PCR. This method, termed InSite™-BAX® System method, can provide results in two days or less. This method was confirmed to provide accurate, rapid results by using plastic surfaces contaminated with both high and low levels of *Listeria monocytogenes*, strain DD1307, and a negative control organism, *Pseudomonas aeruginosa* and comparing results to the standard reference methods.

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The two-part process starts with InSite™ *Listeria*. The premoistened InSite *Listeria* swab was used to thoroughly swab each test area. The swab was then placed back into the swab tube, held at room temperature for 2 hours and then activated by breaking the internal seal so all liquid could flow down into the tube containing enrichment media. The sealed tube was then incubated at 37°C for 24-48 hours, with visual inspection at 24 and 48 hours for a color change of the medium. Small volumes were pulled from the incubating devices and tested with the BAX® System. Multiple BAX® System assays were run including Real-Time Genus *Listeria* and Real-Time *L. monocytogenes* assays, standard PCR assays for *Listeria* species and *L. monocytogenes* and 24E *Listeria* and *L. monocytogenes* assays. Each sample was again confirmed by culture regardless of the BAX® System results following USDA MLG Chapter 8.11.

The InSite *Listeria* devices demonstrated a positive color change to grey/black for 16/20 low-level *Listeria* samples and 5/5 high-level samples after 48 hours. These presumptive positive samples also fluoresced green when exposed to UV light verifying the presence of *L. monocytogenes*. When an aliquot from the devices were tested at 24 and 48 hours with the BAX® System, all 8 PCR assays were in 100% agreement with the InSite *Listeria* test results. All results were identical to culture methods. The corresponding samples enriched using the USDA FSIS reference method were also tested with all BAX® System PCR assays, returning positive results for 12/20 low-level *Listeria* samples 5/5 high-level samples, identical to culture.
The results of the InSite™ *Listeria* and BAX® System method was compared to the results of the USDA FSIS reference method. The results of these statistical analyses demonstrate no significant difference between the methods. This demonstrates the combined ability of the InSite *Listeria* environmental test swab and the BAX® System to accurately detect and confirm the presence of *Listeria* from plastic surfaces after 48 hours.

The utility of this accurate, rapid method can be used to reduce testing costs by screening the environment first with the InSite *Listeria* and then confirming all presumptive positives that exhibit a grey/black color change with any of the BAX® System PCR assays for Genus *Listeria* and *L. monocytogenes*. Added values are reduced time to results and accuracy, allowing release of product more quickly and helping with inventory flow.

**Conclusion**

*Listeria* contamination can affect not only the consumer but also your food organization. The risks of illness and potentially death are not worth the cost of a recall, a plant shutdown or a lawsuit. As more methods for *Listeria* detection are developed, a balance will need to be maintained between simple, rapid cost effective, screening methods with an acceptable presumptive positive rate for environmental samples (and primary preventative control) and the more definitive confirmation methods for finished product testing. The InSite™-BAX® System method achieves both while reducing overall food processing facility operational costs.