

CASE STUDY

A practical strategy for *Listeria* control measures customized to individual manufacturing operations

The Need for *Listeria* Mapping

As proponents of food safety, manufacturers are proactively interested in understanding the ecosystems of their production facilities. As a result, manufacturers need a way to implement a more robust environmental monitoring program by inclusion of subtyping bacteria to determine whether there is persistent *Listeria* within their facilities. Transient *Listeria* are temporary and with proper sanitation practices should be eliminated; whereas resident or persistent *Listeria* may cause large-scale issues within a manufacturing facility, increasing the risk for *Listeria* contamination of the product, and indicating that corrective actions have not yet eliminated the root cause of the facility contamination. Current practices of routine presence/absence testing of environmental samples do not identify if there are persistent or resident *Listeria* within a facility.

The U.S. Food and Drug Administration (FDA) has increased focus on finding resident strains in food facilities through collecting large numbers of environmental samples and characterizing the isolates using whole genome sequencing (WGS). Currently, the routine use of WGS is not practical for facilities to implement in preparation for FDA visits due to cost and time factors. Manufacturers need a way to collect data quickly to know when persistent or transient strains are present in their facilities so they may take decisive action.

Rheonix *Listeria* PatternAlert™ assay provides an industry solution to these problems. When the *Listeria* PatternAlert™ assay is used in an environmental monitoring program, it can determine the presence of persistent or temporary patterns, thereby giving an understanding of a facility's eco-system. The data provides a clear strategy for *Listeria* control measures specific to each plant's environment and allows for faster adjustment of standard sanitation operating procedures (SSOPs) to reduce the risk of finished product contamination.

About Rheonix PatternAlert™ Technology

The *Listeria* PatternAlert™ assay tests for the presence or absence of genomic sequences present in *Listeria* strains, using 15 targets on a low-density DNA microarray. Each sample is scored on the presence or absence of each spot, resulting in a "pattern" that encompasses a group of strains. This pattern represents a molecular signature that can be compared against a user-specific database to determine where and/or when that pattern has been seen before. This pattern data is unmatched to any regulatory or public health database because it is not WGS or pulsed field gel electrophoresis (PFGE) data.

The *Listeria* PatternAlert™ assay can be used directly from an enriched environmental sample or an isolate. Testing directly from enrichment saves

tremendous time, as it generally takes several days to obtain a confirmed *Listeria* isolate. This valuable pattern information is available much sooner than when traditional subtyping methods are used, enabling quicker decisions and actions. In addition, the *Listeria* PatternAlert assay enables identification of recurring populations of *Listeria* in facilities that other typing methods may miss, due to those methods' reliance on isolates in pure culture.

About the Study

Mérieux NutriSciences collaborated with manufacturers to design a study to understand the practical application of the Rheonix *Listeria* PatternAlert™ technology. For a three-month period, *Listeria* environmental sample enrichments were collected and analyzed by Mérieux NutriSciences using the PatternAlert™ technology to identify different *Listeria* patterns specific to each facilities' environment.

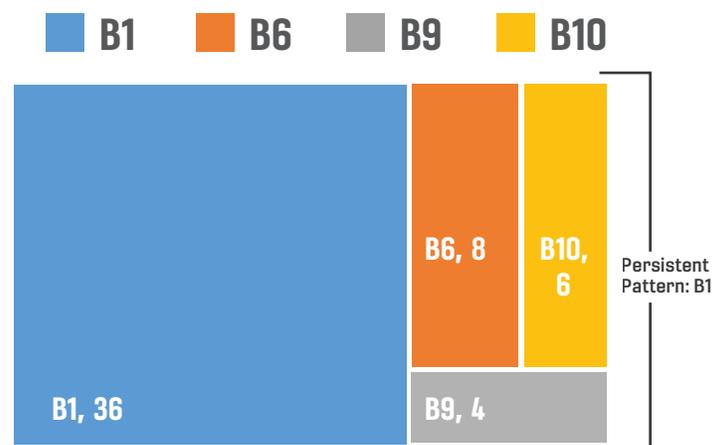
The data was evaluated to understand the frequency of sample patterns, determine root causes in investigations, and consider alternative sanitation measures.

Results from the analysis were available at roughly half the cost and time of traditional subtyping methods and produced fast, actionable data to select and verify the most effective corrective actions.

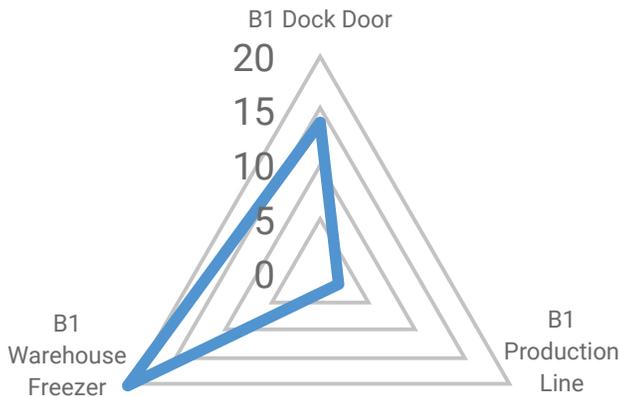
Evaluating the Results

The results of the study detected four different *Listeria* patterns at various prevalence levels within one of the manufacturing facilities. One observed pattern, named B1, presented as a persistent pattern with a prevalence of 67% of positive *Listeria* events.

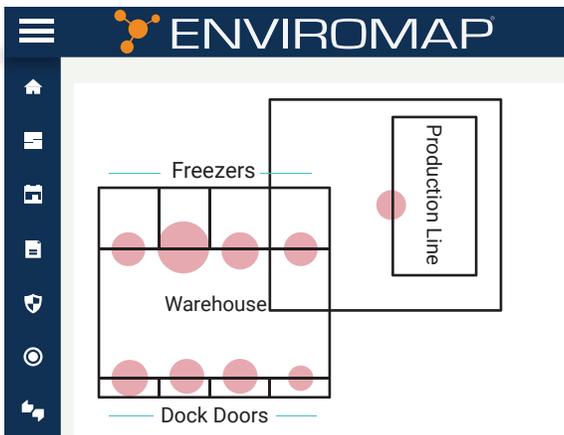
Facility *Listeria* Pattern Prevalence



As Pattern B1 presented as persistent, it was reviewed in detail to understand the source. It was observed 14 times on the floor near the dock door in the warehouse and 20 times near the floor by the warehouse freezers. The pattern was observed to be spreading, and was encroaching near one of the production lines.



When plotting out the prevalence of the persistent pattern in the facility, using a visualization software tool such as Mérieux NutriSciences' EnviroMap, we can see the source of the pattern stemming from the warehouse. As a result, smart, targeted action can take place to address the issue.



Turning Data Into Meaningful Action

The practical application of the data led to the following actions to reduce the occurrence of the persistent pattern:

1. An in-depth review of the warehouse floors for visible cracks, wearing, and unsealed areas around dock doors and freezers. These spots are known harborage areas for *Listeria* growth.
2. An evaluation of the flow into the freezers by workers (warehouse vs. production) to prevent cross-contamination. Separation of staff from high and low risk area prevents spread of bacteria.
3. Installation of sanitizing footbath mats and foamers at the entry points to production.

These actions served as proactive measures to help control *Listeria* within a

manufacturing facility. Powerful subtyping technology like the Rheonix *Listeria* PatternAlert™ assay enables food producers and manufacturers to make such actions more quickly and decisively. This exciting new technology provides manufacturers with the tools they need to guard against *Listeria*.

About Mérieux NutriSciences

Mérieux NutriSciences is a leading global food safety and quality partner — offering chemistry and microbiology testing, labeling, auditing, consulting, sensory testing, customized training, research services, and digital solutions to the food and nutrition industry. Focused on customer excellence, we protect consumers' health through nutritional research, scientific excellence, and innovation. We customize our services to meet the needs of individual manufacturers, food processors, caterers, restaurants, and retailers. Headquartered in Chicago, Mérieux NutriSciences has grown from a single laboratory to have a global presence. Present in 25 countries, Mérieux NutriSciences employs 7,000 people worldwide working in just over 100 laboratories.

About Rheonix

Rheonix has developed the suite of Encompass workstations, fully automated systems that provide highly multiplexed sample-to-answer molecular testing for use in clinical, research and applied testing laboratories. With minimal hands-on time, the Encompass systems offer true walkaway simplicity. Rheonix's growing portfolio of multiplexed food and beverage testing solutions includes the Beer SpoilerAlert™ assay, the most comprehensive beer spoilage panel available; the *Listeria* PatternAlert™ assay, a rapid method for identifying patterns of molecular targets present in *Listeria* direct from an enriched sample; and the NGS OnePrep™ solution, a fully integrated and automated DNA extraction and library prep solution. With Rheonix, getting more information from your sample has never been easier.

