

DuPont Qualicon BAX[®] System

8-HOUR LISTERIA ASSAY

WHITE PAPER

New Use of Proven Technology Enables Faster Detection of *Listeria*

Listeriosis is a serious infection caused by eating food contaminated with *Listeria monocytogenes*. According to the U.S. Centers for Disease Control and Prevention (CDC), an estimated 2,500 people become seriously ill with listeriosis each year in the United States. Although this frequency rate is much lower than for other foodborne illnesses, listeriosis has a fatality rate of 20 percent. Thus, approximately 500 people die annually in the United States due to listeriosis.

For most healthy adults and children, listeriosis is a rare occurrence that causes few, if any, symptoms. Those at risk for serious illness and possible death include: pregnant women; newborns; the elderly; persons with cancer, diabetes or kidney disease; and those with compromised immune systems.

According to the CDC, pregnant women are approximately 20 times more likely to get listeriosis than healthy adults, and AIDS patients are nearly 300 times more likely to contract this infection than people with normal immune systems.

Over the past 20 years, the majority of listeriosis outbreaks have been traced to contaminated ready-to-eat meat products. Some of these involved millions of pounds of food. In addition to the toll on human life, these outbreaks resulted in significant financial losses and severe damage to the reputations of the companies and brands involved.

Clearly, preventing listeriosis is a major concern.

Understanding *Listeria*

There are six different species in the genus *Listeria* -- *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. grayi**. Only *L. monocytogenes* is consistently associated with human illness, having been identified as the cause of listeriosis in humans.

Even though not all *Listeria* are pathogenic, they are considered indicator organisms, so the presence of any *Listeria* in a food plant environment suggests the probable presence of pathogenic species.

Listeria is found in soil and water, and can be carried by animals that appear to be healthy. It has been found in a wide range of foods, including: uncooked meats; raw vegetables; processed foods, such as soft cheeses and deli meats; raw milk products; liquid whole eggs; smoked fish; and a host of ready-to-eat foods.

Pasteurization and cooking effectively kill *Listeria* in most foods. However, in some ready-to-eat products, such as hot dogs and deli meats, contamination can occur between cooking and packaging. It is important to note that *Listeria* can multiply slowly in a refrigerator and can even survive freezing. It is also resistant to salt, nitrite and acid. For at-risk individuals, thorough cooking just prior to consumption is the only sure way to eliminate this contaminant.

*Although *L. murrayi* was once considered a separate species, it has been re-classified as a member of the *L. grayi* species. (See Rocourt, J., et al. 1992. Assignment of *Listeria grayi* and *Listeria murrayi* to a single species, *Listeria grayi*, with a revised description of *Listeria grayi*. Int. J. Syst. Bacteriol. 42:171-174.)

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The miracles of science™

Managing risk requires early detection

Most food producers and essentially all ready-to-eat meat manufacturers in the United States conduct environmental testing for *Listeria*. Common harborage sites – and the cause of most large outbreaks – include HVAC systems, drains and pieces of equipment, such as conveyor belts.

Conducting environmental testing typically allows manufacturers to detect problems faster and take necessary corrective actions sooner than conducting product testing. Another advantage is that even in a very bad situation, only approximately 10 percent of packaged products will be contaminated at a detectable level. Therefore, greater numbers of packaged products must be tested in the hopes of detecting contamination before product is released for distribution.

Numerous detection methods for environmental testing are currently available – from standard culture techniques and lateral flow processes to immunoassays and PCR-based tests. None of these existing methods can deliver results in less than one day, and some take as many as four to six days.

Ideally, results should be available in less than eight hours so that trouble spots can be targeted during the same shift that the samples were taken and corrective actions can begin the same day.

Innovative use of proven technology

Recognizing the industry's need for a faster detection method for environmental testing of *Listeria*, DuPont Qualicon researchers and scientists took on the challenge. The result is a new genetic-based assay that uses reverse-transcriptase PCR to detect all species of *Listeria* on environmental surfaces, with results available eight hours after sampling.

Reverse-transcriptase PCR has been used successfully in the clinical and diagnostic industries for many years. However, this new PCR assay for the BAX® System is the first commercial use of reverse-transcriptase technology for detecting bacteria in food.

Much like DNA, bacteria possess specific RNA sequences that are unique to a targeted organism. Although each bacterial cell has one or perhaps a few DNA molecules, it can typically contain thousands of RNA molecules.

Reverse-transcriptase PCR is used to initially synthesize complimentary DNA from RNA strands. The process then segues into standard DNA amplification and detection. The huge numbers of RNA fragments present at the start of the PCR reaction lead to dramatically improved sensitivity vs. other detection methods. In fact, reverse-transcriptase PCR can detect *Listeria* species at extremely low concentrations (<10 cfu/mL, except *L. grayi* at <50 cfu/mL).

What's more, with reverse-transcriptase PCR, samples do not require the usual one or two days' enrichment in nutrient broth. Instead, *Listeria* cells are resuscitated by heating in the collection buffer solution for a few hours. This provides a "jump start" to the process, enabling results in just eight hours.

Flexibility accommodates work flow

With a reliable 8-hour *Listeria* assay, food companies that test in-house can get results by the end of the day. This allows for immediate intervention and clean-up, thus reducing the risk of product contamination to just one shift.

And companies that send their samples out to third-party labs can also get results much faster. The flexibility of the 8-hour assay allows for extended hold times, so labs that receive samples in the afternoon can refrigerate them overnight and test in the morning, delivering fast, next-day results,

Convenient, easy-to-use assay

In addition to speed and accuracy, the new BAX® System 8-hour *Listeria* assay offers convenience and ease of use. No special skills are required and the PCR tablets eliminate the extra pipetting steps needed for manual RNA extraction. What's more, clear and reliable "yes-no" answers virtually eliminate the need for expert interpretation of results.

The collection kit contains specially designed environmental wipes that enhance sample collection-and-release, and optimized buffers that help to improve sensitivity. The collection kit, which also contains sterile gloves, can be shipped separately from the assay kit, making it easy to collect samples at many sites and send to a central laboratory for testing.

The use of melt curve detection allows this assay to be run on either the original or newer Q7 BAX® instrument. And, LIMS-compatible data allows for easy storage, sharing and retrieval.

For more information

The BAX® system 8-hour *Listeria* assay has been certified by AOAC-RI as Performance Tested Method #030801. To learn more about how this assay can help you reduce risk throughout the production environment, contact your DuPont Qualicon account manager or call 1-800-863-6842.